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SIGNIFICANCE OF HEPARIN IN THE PROCESS OF BLOOD COAGULATION

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Heparin is one of the most important anticoagulants of blood. The use of heparin as an anticoagulant presents many advantages over other preparations; it is readily soluble in water and physiological saline, its action is virtually instantaneous (peak effect after 5 minutes), it is rapidly eliminated from the organism, and it does not increase the osmotic pressure of blood. Yet, although heparin is so widely applied, the mechanism of its effect on blood clotting has not been adequately studied. Heparin is usually regarded as a physiological anticoagulant, which is always present in the blood, and which inhibits intravascular clotting by binding one [7, 11, 13, 14, 16] or more [9, 10] of the components of the blood clotting system.

The present paper is devoted to the study of the reaction between heparin and thrombotropin, which was shown by B. A. Kudryashov [4] in 1948 to be a new component of this system. Thrombotropin is the initiator of the process of blood clotting, its action on prothrombokinase being the first step of this process. The effect of heparin on this important stage of blood coagulation has not heretofore been investigated.

We also investigated the reactions of heparin with blood calcium, prothrombokinase, and thrombokinase; very diverse views as to the nature of these reactions have been reported, and highly contradictory opinions have been expressed by different authors.

EXPERIMENTAL METHODS AND RESULTS

We used the following methods: determination of blood prothrombin activity [5], of thrombotropin [4], of blood calcium [de Waard (3)], clotting time of normal and heparinized plasma (G. G. Bazazyan's method) after addition of thrombin. In a number of experiments we used plasma which had been treated with $Ca_3(PO_4)_2$, to remove prothrombin [15] and thrombotropin [1].

Heparin and thrombotropin: we studied the mutual relations of heparin and thrombotropin in blood clotting systems, in vivo, using albino rats, and in vitro, with thrombotropin preparations.

We examined the effect of thrombotropin on the activity of heparin added to $Ca_3(PO_4)_2$ -treated rat plasma to which thrombin had been added to promote clotting. These experiments were repeated with fibrinogen solutions instead of plasma. Thrombotropin was added to the plasma or fibrinogen solutions simultaneously with 0.5% heparin.

The activity of heparin fell in the presence of physiological levels of thrombotropin activity; this was shown by a shortening of clotting time of thrombin-activated plasma, from 25 to 17.8 seconds. Thrombotropin alone had no effect on the clotting time of plasma after addition of thrombin.

Only active thrombotropin exerted any effect on the activity of heparin. If the thrombotropin was inactivated by heating at 48° for 5 minutes it did not lower the activity of heparin, and even somewhat lengthened the clotting time of plasma treated with thrombin.

The same effects were observed when 0.5% heparin and thrombotropin solutions were injected simultaneously

into the jugular vein of rats; active thrombotropin shortened the clotting time of heparinized plasma treated with thrombin, from 30.8 to 20.3 seconds, while inactivated thrombotropin somewhat lengthened clotting time, from 30.8 to 35.9 seconds (30.8 seconds is the mean clotting time found for thrombin-induced clotting of plasma from control rats injected with 0.5% heparin solution and physiological saline).

The effect of 0.5% heparin solution on the thrombotropin activity of rat plasma was studied in vitro and in vivo. The in vivo experiments, performed on 33 rats, showed a mean thrombotropin activity of 94.3% before injection of heparin, and of 25.1% after. The same volume of 0.85% NaCl was given to a group of 12 control rats, instead of heparin solution, and in this group thrombotropin activity fell very slightly, from 96.5 to 85.5%, i.e., it remained within the normal range.

It thus appears that heparin causes a marked fall in thrombotropin activity. The same effect was seen in experiments in which 0.5% heparin was added to rat plasma in vitro. Similar results were obtained when fibrinogen solutions were taken instead of plasma.

Effect of Prothrombokinase and Thrombokinase on the Activity of Heparin

The effect of heparin and thrombokinase taken together was studied separately for thrombokinase itself and for its precursor prothrombokinase. Prothrombokinase was activated by means of plasma thrombotropin, using B. A. Kudryashov's method [4].

Heparin was dissolved in prothrombokinase or thrombokinase solutions, to give a concentration of 0.5%, and 0.1 ml of the resulting solutions was added to 0.9 ml of $Ca_3(PO_4)_2$ -treated rat plasma.

Clotting of 0.2 ml portions of the mixtures was effected by adding an equal volume of thrombin solution, and clotting time was recorded. As a control, we added the same volume of heparin solution dissolved in 0.85% NaCl, instead of in prothrombokinase or thrombokinase solutions, to plasma.

It was found that prothrombokinase or thrombokinase somewhat lowered the activity of heparin, shortening the clotting time from the control value of 15 sec. to 9-10 sec.

There was no significant difference between the effects of prothrombokinase and thrombokinase.

Addition to the plasma of prothrombokinase or thrombokinase alone had no effect on the clotting time.

We used prothrombokinase prepared from rat brain, thromboplastin, and a preparation of prothrombokinase not contaminated with other proteins, called Hemostasin. Hemostasin was somewhat more active than thrombokinase or prothrombokinase prepared from rat brain.

Heparin and Calcium

In the first series of experiments we examined the effect of heparin on the concentration of calcium salts in rat blood, both in vivo and in vitro, while in the second series we examined the effect of adding calcium chloride on the activity of heparin.

In the first series of in vitro experiments blood was taken from the jugular vein of rats, into a syringe containing 0.1 ml of 0.5% heparin solution, and it was then centrifuged, and the calcium content of the heparinized plasma was determined. The mean value found was 7.62 mg %. Blood had been taken previously from the same rats, with 0.85% NaCl in the syringe instead of heparin solution, and the calcium content of the serum was found to be 7.32 mg %.

It follows that the calcium content of blood is unaffected by heparin in vitro.

The same result was obtained in experiments in which heparin was injected into a vein, in various amounts, and blood samples were taken after injection.

We examined the effect of calcium chloride on the activity of heparin, using whole oxalated rat plasma, $Ca_3(PO_4)_2$ -treated rat plasma, and fibrinogen solutions. We hoped in this way to avoid the contradictory results obtained by workers using one type of experimental material only – plasma or fibrinogen.

For the in vitro experiments we added to 0.9 ml of plasma or fibrinogen 0.1 ml of 0.5% heparin solutions in 0.025 M calcium chloride, and clotting of the mixture was effected by means of thrombin solution. The control systems consisted of 0.9 ml of the same plasma or fibrinogen with 0.1 ml of heparin dissolved in 0.85% NaCl.

Our experiments showed, as did those of M. A. Ukolova (6), that calcium chloride causes considerable lowering of the activity of heparin (clotting time was shortened from 20.9 to 12.6 seconds for whole plasma, from 23.4 to 12.3 seconds for $Ca_3(PO_4)_2$-treated plasma, and from 12.0 to 6.7 seconds for fibrinogen solution). Addition of calcium chloride alone to the same plasma or fibrinogen solution had no effect on clotting time after addition of thrombin.

In the in vivo experiments heparin was injected, followed after 5 minutes by 0.025 M $CaCl_2$ solution. The same volume of physiological saline was in injected into animals of the control series. Blood samples were taken 5 minutes after injecting calcium chloride or saline solution, the plasma was separated by centrifugation, and was coagulated by means of thrombin. It was found that the clotting time of heparinized plasma was shortened from 78.6 seconds after saline injection to 29.2 seconds after calcium chloride injection. Injection of calcium chloride alone had no effect on the clotting time of thrombin-treated plasma.

DISCUSSION OF RESULTS

We conclude from our results that heparin and thrombotropin are antagonists. Addition to rat blood, in vivo or in vitro, of thrombotropin preparations, at levels close to the physiological ones, lowers the activity of heparin added to the same systems. This property of thrombotropin is abolished by thermal inactivations. Introduction of heparin into the blood lowers the activity of added thrombotropin. Heparin has no effect on the calcium content of blood.

Thrombotropin, prothrombokinase, thrombokinase, and calcium do not, by themselves, shorten the clotting time of oxalated rat plasma to which thrombin has been added. This confirms the findings of Conley (8) and Lyttleton (12) that free heparin is either absent from plasma, or present only in very insignificant amount; binding of free heparin by the above-named substances could cause significant shortening of clotting time of thrombin-treated plasma, as has been shown above.

It appears that calcium and active thrombotropin present in blood may exercise a sort of buffer action, preventing heparin from exerting its action in normal physiological states of the organism (i.e., when small amounts of heparin may be entering the blood stream).

The presence of large amounts of heparin in the blood in certain pathological states may be interpreted as a defense reaction of the organism, whereby heparin acts in the first place by binding plasma thrombotropin, so preventing intravascular clotting.

It may thus be concluded that heparin does not play any definitive part in the normal physiological process of blood clotting. In our opinion, the maintenance of the fluid state of blood is ascribable to the absence of thromobokinase from the plasma, but not to the inhibitory action of heparin on prothrombin or other blood constituents, as is generally supposed.

The basic physiological importance of heparin appears to be that it is secreted into the blood stream in large amounts when thrombogenic components appear, and could directly lead to intravascular clotting; heparin powerfully inhibits their activity.

Consideration should be given to the treatment of hyperheparinemia by administration of calcium chloride, which powerfully inhibits the activity of heparin. In using heparin therapeutically account should be taken not only of prothrombin, but also of thrombotropin, activity.

SUMMARY

The relationship of anticoagulant heparin was investigated with thrombotropin, prothrombokinase, thrombokinase and calcium.

It was demonstrated that heparin and thrombotropin are in antagonistic relationship.

Prothrombokinase and thrombokinase reduce the activity of heparin to the same extent.

Calcium chloride greatly decreases the activity of heparin introduced into the bloodstream.

Heparin introduced into the blood has no effect on the concentration of calcium salts in the blood.

The author considers that heparin exerts a powerful anticoagulant effect with compound action only in a pathological condition of the body when thrombogenic components which may directly cause the intravascular blood coagulation enter the bloodstream.

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PRESENCE OF COMPONENTS OF THROMBOPLASTIN IN ERYTHROCYTES

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Blood coagulation is a complex biological process, requiring the participation of a large number of factors present in the plasma, the blood platelets, and the tissues. A number of workers [1 – 3, 4, 5, 11, 12] have shown that a number of factors take part in the process, other than fibrinogen (Factor 1), prothrombin (Factor 2), thromboplastin (Factor 3), and calcium salts (Factor 4). It is known, moreover, that thromboplastin is present in an inactive form in the blood platelets, leucocytes, and body tissues [1, 8]. Blood thromboplastin originates from a number of factors present in the blood platelets and the plasma. Special importance in the formation of thromboplastin has been ascribed to antihemophilic globulin (Factor 8), to the plasma component of thromboplastin (Factor 9), and to Factor 10. Ac-globulin (Factor 5), pro-convertin (Factor 7), and calcium chloride (Factor 4) appear also to take part in the formation of thromboplastin, since none of these factors has any effect on the activity of formed thromboplastin [9]. Blood thromboplastin originates from the interaction of platelet and plasma factors. The existence of a relatively large number of factors which are active in the first phase is supported by the fact that deficiency of each individual factor, except Factor 4, results in a specific form of hemorrhagic diathesis.

Evidence has been reported in the literature that the thromboplastin activity of blood depends on the presence of fully active blood prothrombokinase (blood platelets), and on the concentration of plasma thromboplastin factors [1, 11]. The more thromboplastin is formed in the first phase of clotting, the more prothrombin is converted into thrombin in the second phase, for conversion of fibrinogen into fibrin in the third phase.

It follows from the above that the most complex phase of blood clotting is that of thromboplastin formation. Very little study has been devoted to the relations of erythrocytes to this process.

A number of authors have established the presence in human erythrocytes of factors participating in the formation of thromboplastin [10, 13].

The present paper presents evidence of the presence of thromboplastin factors in dog erythrocytes.

EXPERIMENTAL

The experiments were performed on male dogs. Four ml of blood were taken from a superficial vein of a forelimb of healthy dogs, without anesthesia; 2 ml were immediately placed in a centrifuge tube containing 0.5 ml of 1.34% sodium oxalate solution, and the remaining 2 ml were placed into another centrifuge tube. Two series of experiments were performed.

In the first series evidence of the presence of thromboplastin factors in erythrocytes was based on measurements of recalcification times of oxalated dog plasma in the presence of aqueous hemolyzates of washed erythrocytes. As controls we measured recalcification times in systems containing physiological saline.

Plasma recalcification time was determined in the following way: 0.2 ml of 0.277% $CaCl_2$ solution and 0.1 ml of hemolyzate were added to a 8 × 100 mm test tube, followed quickly by 0.1 ml of plasma, a stopwatch being started at the same time. The test tube was shaken, and the time of appearance of fibrin floccules

was noted. The measurements were made in a water bath at 38°. Recalcification time was taken to be the interval between introduction of plasma and appearance of fibrin floccules [3].

In the second series of experiments we examined the effect of hemolyzate on prothrombin requirement. The prothrombin requirement for clotting was determined by the method of Quick [14]. The prothrombin requirement was calculated from the difference between its content in the plasma and serum when blood was allowed to clot in the normal way, after addition of hemolyzate or of the same volume of physiological saline (control). The data so obtained served for the estimation of total thromboplastin activity [6].

EXPERIMENTAL RESULTS

Our control experiments showed that the recalcification time of oxalated dog plasma varied within the range 80-142 seconds. This time was shortened when a diluted hemolysate of dog erythrocytes was added. A statistically treated presentation of the results is given in Table 1.

TABLE 1

Effect of Hemolyzate on Recalcification Time of Oxalated Dog Plasma

Statistical coefficients	Plasma recalcification time (seconds)	
	plasma and physiological saline	plasma and red cell hemolyzate
M	100	37
$\sigma\pm$	21	4
$m\pm$	5	1
t		12.6
P		< 0.001

Note: M is the mean arithmetic value, σ is the standard deviation, m the mean error, t is the index of significance of deviations, and P is the probability of difference, calculated in relation to the recalcification time found in the presence of physiological saline.

It appears from the data of Table 1 that when physiological saline is added to the plasma the recalcification time, as calculated from the arithmetic means, is equal to 100 seconds ($\sigma = \pm$ 21 seconds); with red cell hemolyzate this line is decreased to 37 seconds ($\sigma = \pm$ 4 seconds.) Hence the coagulability of oxalated plasma in the presence of hemolyzate and with subsequent recalcification is increased, with reference to the control ($P < 0.001$).

The shortening of recalcification time of plasma in the presence of hemolyzate is related to raised thromboplastin activity of the plasma. This may have been due to introduction of blood platelets together with the hemolyzates, or to the presence of thromboplastin components within the red cells. Special examinations showed that platelets were absent from erythrocyte hemolyzates. It may hence be assumed that the increase in thromboplastin activity of plasma observed after addition of hemolyzate is due to the presence of thromboplastin factors in erythrocytes (Table 2).

The data of Table 2 show that addition of hemolyzate raises the prothrombin requirement, as compared with the control and with normal values ($P < 0.001$), and this points to a raised thromboplastin activity of plasma mixed with hemolyzate. It may hence be concluded that factors possessing thromboplastin activity are present in erythrocytes.

Recalcification time in the presence of hemolyzate was accordingly shortened, and prothrombin requirement raised. It has been shown by a number of workers [1, 12] that the thromboplastin activity of blood is determined by the amount of platelet and plasma factors present, contributing to the formation of thromboplastin. The higher the content of such factors, the higher will be the thromboplastin activity of the blood, and this, in turn, determines the prothrombin requirement for the blood coagulation process. The raised thromboplastin activity and the shortened recalcification time of plasma containing hemolyzate may thus be ascribed to the presence within erythrocytes of thromboplastinogenic components participating in the formation of blood thromboplastin.

TABLE 2

Effect of Hemolyzate on Prothrombin Requirement

Statistical coefficients	Prothrombin requirement (%)		
	under ordinary conditions	in presence of hemolyzate	in presence of physiological saline
M	68	84	65
σ±	2	4	3
m±	0.5	1	1
t		14.4	0.4
P		<0.001	>0.05

Our finding of thromboplastin factors within dog erythrocytes is in agreement with those of Quick et al. and of Ottoviani and Dettori for human erythrocytes. The presence of thromboplastin factors in human and dog erythrocytes supports the view that this may be a general biological phenomenon.

SUMMARY

Data concerning the presence of thromboplastin factors in dog erythrocytes are presented. The presence of these factors was judged by the influence of water hemolyzate of washed erythrocytes on the time of recalcification of the oxalated plasma and on utilization of prothrombin. The average time of recalcification of oxalated plasma equalled 100 seconds ($\sigma = \pm 21$), while utilization of prothrombin was 68% ($\sigma = \pm 2$). Addition of hemolyzate of erythrocytes to the plasma was associated with reduced duration of recalcification ($P < 0.001$). Utilization of prothrombin was increased in presence of hymolyzate ($P < 0.001$).

Reduced time of recalcification and increased utilization of prothrombin are connected with the presence of thromboplastin factors in erythrocytes.

The presence of thromboplastin factors in erythrocytes of human beings and dogs supports the view that this phenomenon has a general biological character.

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EVALUATION OF THE AMOUNT OF PEPSIN AND HYDROCHLORIC ACID SECRETED BY A PAVLOV STOMACH POUCH IN DOGS*

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Curves representing rate of secretion of gastric juice, its acidity, and its digestive power, after feeding dogs with bread, meat, or milk were first derived by I. P. Pavlov, and are familiar to all physiologists. It appeared to us that the secretory function of the stomach might be more accurately assessed from a consideration of the absolute amounts of hydrochloric acid and of pepsin units secreted by the gastric glands, rather than from the values of the acidity of the juice and of its enzymic activity, although the importance of such data cannot be denied. The volume of the juice is in the first place an indication of the amount of water secreted. The volume of juice does not always vary parallel with the amount of hydrochloric acid and of pepsin. The results of our experiments confirm the opinions of those authors who believe that secretion of hydrochloric acid takes place independently of secretion of pepsin.

Although it is desirable to make use of three tests for an appraisal of the secretory function of the stomach (amount of juice, number of milligrams of hydrochloric acid, and number of pepsin units), papers continue to appear in which the authors make use of acidity and peptic activity data. They all come to the conclusion that these two indices do not reveal any regularities whatsoever.

One of the obstacles to the application of the method proposed by us is the lack of any simple and accurate procedure for determining the number of pepsin units. As is known, Mett's method is not suitable for this purpose. N. P. Pyatnitsky (2) suggested in 1937 that peptic activity could be evaluated from the milk-curdling power of the gastric juice. The same proposal was made earlier by V. V. Savich and F. M. Migal [6], on the basis of I. P. Pavlov's view that pepsin and chymosin were identical. At that time, however, the majority of workers ascribed the milk-curdling activity of gastric juice to a special enzyme, chymosin, and, moreover, the milk curdling technique was not sufficiently simple and accurate. It is now believed that chymosin is absent from the gastric juice of adult animals and humans.

S. Buchs [8], who studied the digestion of edestin by hydrochloric acid extracts of the gastric mucosa of animals, in buffer solutions (glycine – hydrochloric acid), found two pH optima, at pH 2 and 4.5. He hence concluded that gastric mucosa contain two enzymes, pepsin and cathepsin. Tolekmitt [11] was unable to confirm the presence of two pH optima for digestion of protein by gastric juice. For this reason he supported the view that the existence of two proteolytic enzymes has not been established, in gastric juice. We believe, on the basis of the results obtained by M. N. Selyukova [7] in our laboratory, that cathepsin is certainly present in hydrochloric acid extracts of gastric mucosa. As for gastric juice, it either does not contain any cathepsin, or there are not more than traces of it.

Northrop's crystalline pepsin exerts a marked milk-curdling action. The milk-curdling activity is proportional to the amount of pepsin.

Instead of whole milk we now use milk diluted with buffer solution, for the evaluation of milk-curdling

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TABLE 1

Curdling time (minutes)	Compared with normal	Approximate number of units per 1 ml	Recording of results
Less than 3	Very strong	More than 2500	++++
3—4	Moderately strong	2001—2500	+++
4—10	Normal	1001—2000	++
10—15	Weak	501—1000	+
More than 15	From zero to traces	0— 500	0 or traces

activity. In a study of the rate of conversion of pepsinogen into pepsin, made in 1933, Ege and Menk-Thygesen [9] used acetate buffer at pH 5 (42 g of sodium hydroxide and 115 ml of 80% acetic acid per 1000 ml of water). This buffer was added in equal volume to fresh cow's milk. Five ml of milk-acetate mixture was added to 0.1 ml of gastric juice in a test tube, and the time required, at 25°, for the appearance of floccules of casein on the walls of the test tube was recorded.

Kleiner [10] considers that rennin is absent from the gastric juice of human adults, and, similarly to us, considers that milk-curdling activity can be taken as a measure of pepsin activity. As he considered that the method of pepsin assay previously devised by Borowski, Tauber and Kleiner was too complicated, Kleiner worked out a simpler modification of this method. Gastric juice is diluted 1:50 with water, and 1 ml of the solution is added to 10 ml of milk-acetate mixture at 20°, the mixture is placed in a water bath at 20°, and the number of minutes required to curdle the milk is recorded. The results are read according to Table 1.

As is evident from Table 1, the determination of activity of gastric juice in Kleiner units is very approximative and inconvenient.

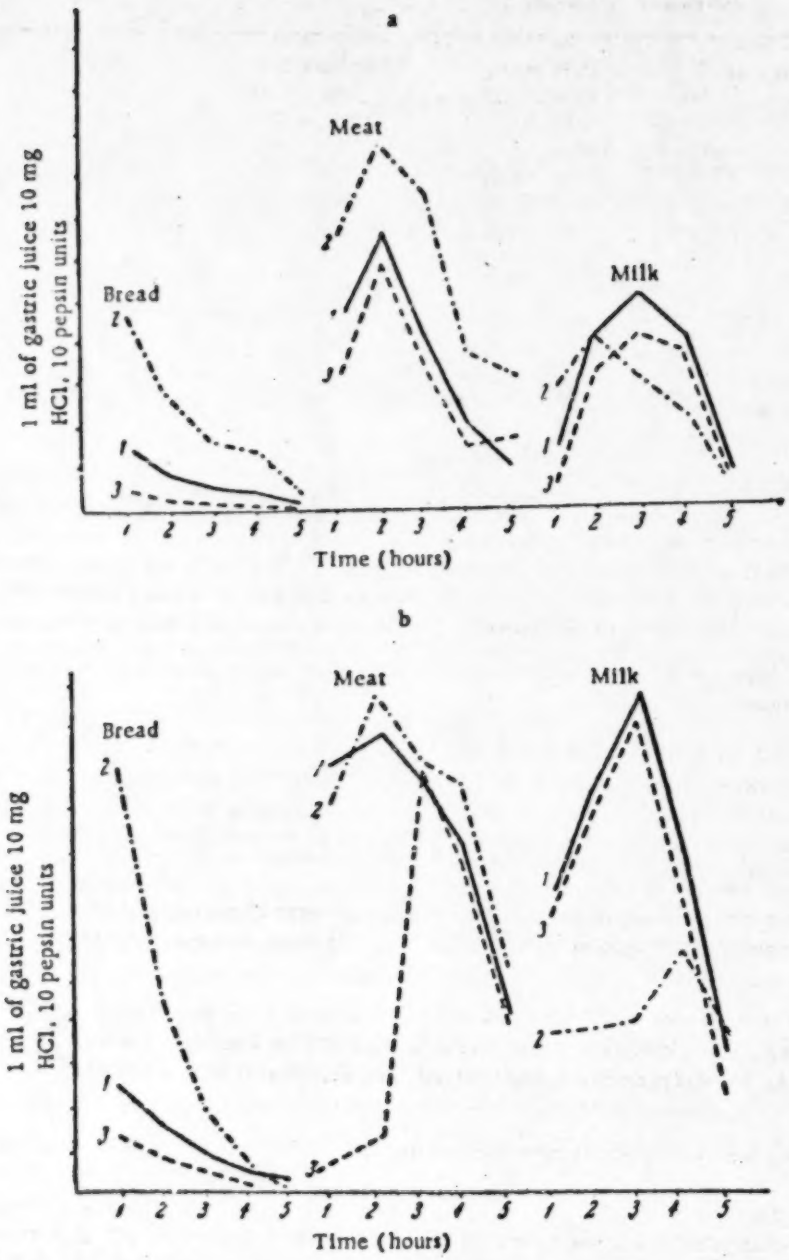
N. P. Pyatnitsky suggested in 1954 that a unit of pepsin should be taken as that amount which causes curdling of 5 ml of milk-acetate mixture in 60 seconds at 25°. Dividing 60 by the number of seconds required with a measured aliquot of gastric juice gives the number of pepsin units in the aliquot. For example, if 0.1 ml of undiluted juice curdles 5 ml of milk-acetate mixture in 20 seconds, 1 ml of juice will contain 30 pepsin units ($10 \times 60/20 = 30$).

The simplicity and accuracy of the chymosin method of assay of pepsin, and the convenient units for expression of its amount, encouraged us to repeat I. P. Pavlov's experiments on dogs fed with bread, meat, and milk.

We had four healthy dogs with Pavlov pouches at our disposal. We performed three experiments with each type of feeding. The secretory response varied individually for each dog, but was always the same for a given sort of food. For this reason, the results of only one experiment with a particular food are presented in Table 2.

The following regularities are evident from an inspection of the curves for total secretion, secretion of hydrochloric acid, and secretion of pepsin, plotted from our experimental results. After a bread meal (200 g) very little gastric juice and hydrochloric acid are secreted, but relatively large amounts of pepsin (see Figure). Much juice, hydrochloric acid, and pepsin are secreted in response to meat (200 g). Ingestion of 600 ml of milk leads to a smaller secretion of juice, hydrochloric acid, and pepsin than after meat. In all the experiments, the amount of acid secreted varies in the same direction as does the volume of juice and its pepsin content, although full parallelism is not observed. Secretion of acid and of pepsin also do not always vary parallel. As is known, the activity and the acidity of the juice vary reciprocally as a general rule: the higher the acidity of the gastric juice of a given dog, the lower is its peptic activity. The acidity of juice is lowest at the beginning and the end of the secretory period, and remains fairly constant at the height of secretion.

It follows from our observations that there is no strict parallelism between secretion of water, pepsin,



Secretion of (1) gastric juice; (2) pepsin; (3) hydrochloric acid, in response to bread, meat, and milk.
a) by the dog Palma, b) by the dog Laska.

TABLE 2

Indexes of the Work of a Pavlov Gastric Pouch Over a 5 Hour Secretory Period

Hour	ml of gastric juice	Activity in ml 0.1 N NaOH			Activity	HCl	Pepsin units	
		free	bound	total	1 ml	mg		
Dog Palma								
1	3	10	30	50	15	4.4	45	Fed 200 g of bread
2	2	18	28	58	13.3	3.4	26 6	
3	1.5	10	34	50	11	2.4	17.5	
4	1	6	37	50	13.5	1 5	13.3	
5	0.8	0	38	46	7.5	1.1	6	
Total amount of juice over 5 hours	8.3					12.8	108.4	
1	9	70	30	108	7.5	33	67	Fed 200 g of meat
2	13	100	25	132	6.6	59	85	
3	8	82	30	122	9.2	32	73	
4	4	60	32	104	9.4	14	36	
5	2.2	10	30	52	15	15	33	
Total amount of juice over 5 hours	36.2					153	294	
1	2.8	22	20	80	10	4.3	28	Fed 600 g of meat
2	8	80	27	120	5	31.4	40	
3	9.8	90	30	130	3.1	39.2	30 3	
4	8	93	28	129	2.7	35.2	21.6	
5	2	30	20	60	3.1	3.6	6.2	
Total amount of juice over 5 hours	30.6					113.7	126.1	
Dog Ryzhik								
1	1.2	10	50	80	8.5	2.6	10.2	Fed 200 g of bread
2	1	10	40	60	12	1.8	12	
3	0.9				20		18	
4	0.8				15		12	
5	0.5				13.3		6.6	
Total amount of juice over 5 hours	4.4					4.4	58.8	
1	4.5	40	30	90	12	10.5	54	Fed 200 g of meat
2	5	45	30	92	10	13.6	50	
3	3	20	40	72	8.3	6.6	24.9	
4	2.5	10	35	50	7	4.1	17.5	
5	2		30	40	8.5	2.2	17	
Total amount of juice over 5 hours	17					37	163.4	

TABLE 2 (continued)

Hour	ml of gastric juice	Activity in ml 0.1 N NaOH			Activity	HCl	Pepsin units	
		free	bound	total	1 ml	mg		
1	3	30	50	98	11.5	8.7	34.5	Fed 600 g of meat
2	3.5	40	50	100	10	11.8	35	
3	4	50	30	89	6.6	11.7	26.4	
4	2	40	10	69	10.8	3.6	20.6	
5	1				12		12	
Total amount of juice over 5 hours	13.5					35.8	128.5	
Dog Dik								
1	3.5	8	16	52	14.3	3	50	Fed 200 g of bread
2	2.5	4	18	40	12	2	31	
3	1		12	22	8.5	0.4	8.5	
4	1				7.5		7.5	
5	0.5				6.6		3.3	
Total amount of juice over 5 hours	8.5					5.4	100.3	
1	9	98	10	112	6.6	34	59.4	Fed 200 g of meat
2	11	102	12	120	5.7	45.7	62.7	
3	8	90	18	116	9	30.7	72	
4	4	80	20	102	15	14.6	60	
5	1.5	40	30	80	20	3.4	30	
Total amount of juice over 5 hours	33.5					128.4	284.1	
1	3	30	30	102	8.5	6.6	25.5	Fed 600 g of meat
2	8	60	30	120	7.5	27	59	
3	11.5	80	30	130	6.7	26.1	77	
4	7	80	28	118	9	28.2	63	
5	2	30	20	60	12	3.6	24	
Total amount of juice over 5 hours	31.5					91.5	248.5	
Dog Laska								
1	5	53	22	80	20	13.6	100	Fed 200 g of bread
2	3	64	22	96	15	9.4	45	
3	2	40	16	50	10	4	20	
4	1	20	10	40	6	1.1	6	
5	0.3				5.4	—	1.6	
Total amount of juice over 5 hours	11.3					28.1	172.6	

TABLE 2 (continued)

Hour	ml of gastric juice	Activity in ml 0.1 N NaOH			Activity	HCl	Pepsin units	
		free	bound	total	1 ml	mg		
1	20	100	26	140	4.5	9.2	90	Fed 200 g of meat
2	22	130	15	150	5	11.6	115	
3	19	120	20	150	5.3	97	100	
4	16	110	23	140	6	78	96	
5	8	100	20	125	7.5	30.6	52.5	
Total amount of juice over 5 hours	85					413.6	453.5	
1	14	100	25	132	2.7	63.8	37.8	Fed 600 g of meat
2	19	110	18	136	2	85.7	38	
3	23	116	12	140	1.7	107.4	39	
4	16	112	12	130	3.5	72.3	56	
5	6	70	25	102	6	20.7	36	
Total amount of juice over 5 hours	78					349.9	206.8	

and acid by the gastric glands. The work of these glands cannot be appraised on the basis of the proteolytic activity of the juice, or of its acidity; it may be evaluated from the amount of pepsin and of hydrochloric acid produced during a given secretory period (5 hours). We found that not much juice or hydrochloric acid was secreted in response to feeding 200 g of bread, but that relatively large amounts of pepsin were secreted, the response to 200 g of meat was much juice, much pepsin, and much acid, and to 600 g of milk somewhat less juice, pepsin, and acid than to meat. The proteolytic power of the gastric juice of adult humans and animals, at pH 1.6 – 2, varies parallel with its milk-curdling power (at pH 5). For this reason, the chymosin method for the assay of pepsin merits wide application.

SUMMARY

The authors suggest that absolute quantities of stomach juice, hydrochloric acid and pepsin should be determined during the whole period of secretion for examination of the secretory function of stomach glands. Hemozinic method of pepsin determination, as well as new units of pepsin are proposed.

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THE PHYSIOLOGY OF THE LARGE INTESTINE

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Although there are many works studying the various aspects of digestive tract activity, there are only a few on the physiology of the large intestine. G. B. Berlatsky [2] indicates that the glands of the large intestine produce a small amount of juice. N. D. Strazhesko [6], D. L. Glinsky [4] and others have shown that the intake of food has little effect on the amount of juice secreted. O. V. Varnke and M. M. Levin [3], K. M. Bykov and G. M. Davydov [1] noted that the amount of juice secreted from a fistula of the large intestine fluctuates in both humans and animals. Due to the varying character of this secretion and the small amount of enzymes present, many researchers doubt that the juice secreted by the large intestine is important in the digestive process. How the glands of the large intestine are stimulated has not yet been determined.

EXPERIMENTAL

The experiments were done with dogs. A loop of the large intestine 15-20 cm long, 10-15 cm from the ileocecal valve was partially isolated (according to V. L. Gubar's method [5]). The operation we performed was a circular transection of the mucosa at both ends of the isolated loop, leaving the serous and muscular layers intact. The intestinal sections adjoining each end of the isolated loop were pulled together and anastomosed. A fistula was induced on the isolated loop of the intestine in order to collect the juice, record the motor activity and effect the stimulation of this loop. A fistula was also induced on the stomach. The advantage of this operation was that most of the intramural nerve links were preserved, since the muscular and serous layers of the large intestine's walls had been left intact.

The dogs were fed 16-18 hours before the experiment. Juice was collected from the loop of the large intestine for 4-6 hours; the amount of juice secreted was determined every 30 minutes. We used special stimulants in a series of experiments to stimulate secretion (a suspension of calomel in water, a decoction and paste made from a finely ground beet, a rubber tube and a rubber balloon).

We used a 0.5% solution of novocain with adrenalin added (2-3 drops of a 1:1000 solution) to anesthetize the mucous membrane. In most of the experiments, we recorded the movements of the isolated intestinal loop and the stomach (on a kymograph with an air transmission) while the juice was being collected.

A total of 165 experiments were done.

EXPERIMENTAL RESULTS

The amount of juice spontaneously secreted from the isolated loop of the large intestine in an hour was 0.1-2.5 ml, and 0.5-8.2 ml of juice were secreted in 4-5 hours (Fig. 1, 1).

Secretion did not increase when a small rubber tube was introduced into the cavity of the isolated loop. When a rubber ballon was inflated in the intestinal cavity, secretion increased to 3 ml per hour (Fig. 1, 2).

We anesthetized the mucosa of the isolated loop of the large intestine with 10-15 ml of a 0.5% solution

of novocain In order to determine how stimulation was transmitted from the intestinal cavity of the glands. Anesthetizing the mucosa stopped the secretion caused by the inflation of the balloon, but secretion was resumed (Fig. 1,3) when the anesthesia had passed (after an hour).

The use of natural conditioned and unconditioned stimulants (showing and feeding meat, black bread, etc.) had no material effect on the rate of juice secretion. The inclusion in the diet of rough food stimulants (400 g of boiled beet) did not effect the scretion in the isolated loop of the large intestine either. Nor did stimulating its mucosa with calomel, beet gruel with bouillon, lactose and other substances promote secretion.

We also investigated nocturnal secretion in the dog's large intestine. No nocturnal juice secretion was observed under laboratory conditions, although it was invariably observed under vivarium conditions. A rubber bottle was attached to a cannula in order to collect the juice secreted in the vivarium; up to 10 ml of juice, which had a foul odor and contained a great quantity of mucus, had collected in the rubber bottle after 16-18 hours.

Amylase was determined according to Wohlgemuth, and the amount of dense residue and sol in the intestinal juice was determined. Periodically, we calculated the dense residue of the stool.

The amount of amylase in the juice was not more than 5-10 units. The average dense residue of the juice in 10 experiments was 2.48% (1.28% organic substances and 1.2% inorganic substances). In the dog Foxy, the average dense residue in the stool was from 27.3% to 18.7%.

We found that the motor activity of the large intestine is extraordinarily complex. It consisted of a series of waves differing as to frequency, amplitude and configuration. The waves of the first order (individual contractions) usually occurred 1-2 times per minute (Fig. 2, a). They combined and together formed the complex wave of the second series (Fig. 2, b). The waves of the second order occurred 6-7 times an hour. The pauses between these waves disappeared when, as sometimes happened, the waves of the second order succeeded each other rapidly.

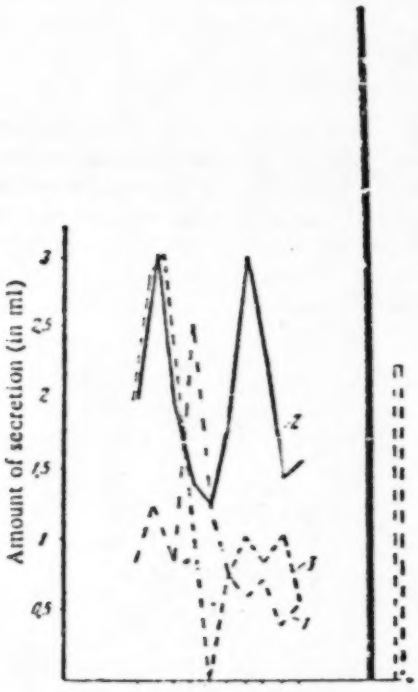

Fig. 1. Secretion from the isolated loop of the large intestine. 1) spontaneous secretion; 2) secretion caused by inflation of a balloon in the cavity of the isolated loop; 3) secretion when mucosa was anesthetized with novocain .

One could also often see small waves of peristalsis on a background of intestinal loop tonic contractions (Fig. 2, c); these could be superimposed upon the waves of the first order.

Motor activity stopped when the mucosa was anesthetized. The contraction waves mentioned, which were recorded by means of a rubber bulb, disappeared.

Comparison of the curves simultaneously recorded from the contraction of the large intestine loop and of the atomach did not allow us to establish any definite interrelation between them.

We injected atropine and pilocarpine in order to find how the secretion and motor activity of the large intestine were influenced by the parasympathetic system.

After an intravenous injection of 1-2 mg of atropine, secretion and motor activity in the isolated loop of the large intestine ceased simultaneously and immediately. When the atropine was injected subcutaneously, a similar effect occurred after 10 minutes. The effect of the atropine lasted $1\frac{1}{2}-2\frac{1}{2}$ hours.

When 3-4 mg of pilocarpine was injected

subcutaneously, the secretory and motor activities of the large intestine became intensified. The contractions increased in frequency, and the amount of juice secreted reached 3.5-8.4 ml an hour.

We then attempted to develop a motor or secretory conditioned reflex of the large intestine to the introduction of atropine into the blood or subcutaneously. We used the sound of a metronome and the injection procedure as the conditioned stimuli and atropine as the unconditioned reinforcement. We could not obtain a conditioned reflex after 60 combinations. However, secretion ceased for a long time after these experiments, and the frequency and amplitude of the contractions were reduced.

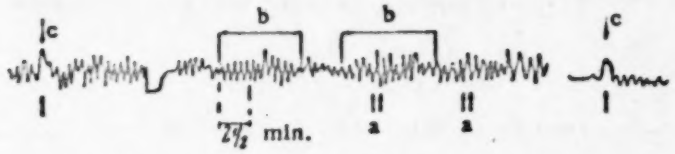

Fig. 2. Motor activity of the isolated loop of the large intestine. a) waves of the first order; b) waves of the second order; c) tonic contractions.

Therefore, mechanical stimulation of the large intestine mucosa is the chief activator of secretion and motor activity in the large intestine. Chemical stimulants have no effect. The parasympathetic nervous system is very important to the realization of the secretory and motor activities of the large intestine.

SUMMARY

It was established in experiments on dogs that secretion of the juice in the large intestine is not constant. The most pronounced reaction is observed on stimulation of intestinal mechanoreceptors. Chemical stimulants, such as calomel, lactose, etc., do not promote secretion. Secretion is present during the night when animals are kept in conditions of vivarium. The motor activity of the large intestine consists of a series of waves which vary in frequency, amplitude and configuration. More rapid contractions (waves of the first order) appear 1-2 times a minute, while the slower ones (waves of the second order) 6-7 times per hour. Secretion and motor activity of an isolated intestinal loop is arrested after novocain anesthesia of its mucous membrane. Contractions increase under the influence of pilocarpine, while the effect of atropine causes their reduction.

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THE EFFECT OF PROPRIOCEPTIVE REFLEXES ON THE HEART. CERTAIN PROPERTIES OF PROPRIOCEPTIVE CONDITIONED-REFLEX INFLUENCES ON THE HEART

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The change in heart activity caused by muscular work has been observed in numerous research works. Individual experimenters [1,4,6,9,10,13], through short experiments, have established proprioceptive influences on the heart, realized through the mechanism of unconditioned reflexes. Recent works have shown the possibility of forming conditioned proprioceptive reflexes on the heart in animals and man [2,3,5,7,8,12,18,19].

We have not, however, found in the literature any systematic description of the properties of the conditioned reflexes whose formation is based on unconditioned proprioceptive reflex influences on the heart.

Therefore, the first task in our study investigating proprioceptive influeces on the cardio-vascular system was to determine the properties of these condioned reflexes.

EXPERIMENTAL

The work was done on five male dogs. The experimental method was as follows. We used a physical (static) load as an unconditioned proprioceptive stimulus, placing the load on the back of a benched dog. The load weighed 80% of the animal's weight. Heart activity was recorded with an electrocardiograph; electrocardiograms in the three standard leads were recorded from the extremities. We used a metronome of 120 beats per minute (M120) as a positive condioned stimulus and a metronome of 60 beats per minute (M60) as a negative. The experiments were done in an ordinary laboratory room and in a conditioned reflex chamber.

Earlier [14], we found that a stable electrocardiogram is established for a given position of a dog's body after the reflexes to environment are extinguished. When no stimuli were used, the dogs' electrocardiograms remained essentially unchanged for over two years after their stabilization. In spite of this, in evaluating various influences on an animal's body, we started with the electrocardiographic background for that day rather than with the already established background electrocardiograms, so that, each time, the experiments began and often concluded with an electrocardiographic recording made without the influence of stimuli. This showed us that, in the dogs, there were respiratory arrhythmia and slight fluctuations in the rhythm, voltage, systolic index and other elements of the electrocardiogram.

In most cases, we used one reinforcement the day of the experiment, occasionally two, and in rare cases, more.

After recording the background electrocardiogram, we tested the effect of the conditioned stimulus alone, then that of the unconditioned stimulus alone, and finally at the end of the experiment, observed the effect of a combination or combinations of the conditioned and unconditioned stimuli. Sometimes, the effect of the conditioned stimulus alone was tested at the end of the experiment. The reinforcement was not used when testing the isolated effect of the positive conditioned stimulus on the animals.

The acting time of the unconditioned stimulus was 4 minutes and, in certain series of observations, 30 seconds and 1-2 minutes. The acting time allotted to the positive conditioned stimulus was, in most cases, the

same as that of the unconditioned stimulus.

We observed changes in the animal heart activity during the imposition of the static load and for 3-4 minutes after removal of the load. Having established this fact, we recorded electrocardiograms 30 seconds, 1, 2 and etc. minutes after the stimulating action had begun and 30 seconds, 1, 2, 3 and 4 minutes after the action of the stimuli had ceased, in order to trace the dynamics of heart activity change. Therefore, the use of the next stimulus was only possible 5 minutes after the action of the preceding one had ceased.

EXPERIMENTAL RESULTS

After having established stable electrocardiograms for the dogs, we developed conditioned reflexes based on the proprioceptive unconditioned reflexes on the heart.

Under conditions of an ordinary laboratory room, a positive conditoned reflex on the heart was formed after 6-10 combinations of the conditioned and unconditioned stimuli (Fig. 1), while fewer combinations of the stimuli were needed to form the conditioned reflex under conditions of a conditioned reflex chamber.

The clearest index of the heart activity changes caused by the static load was the change in rhythm; the number of cardiac contractions per unit of time increased considerably, and the respiratory arrhythmia usually disappeared. Other electrocardiogram elements also changed: the voltage of the positive waves (P and R) first decreased, then increased (at the moment when the conditioned stimulus fully reproduced the effect of the physical load); the voltage of the negative waves (Q and T) decreased; the width of the P wave first increased, but returned to its original size as soon as the conditioned reflex was fixed; the size of the sector P-Q now increased, now decreased, finally becoming shorter; the complex QRS became broader and the interval R-T shorter; the electric ventricular systole decreased by 0.04-0.05 seconds (from 0.22 to 0.17-0.18 seconds), and the systolic index grew due to the reduction of the distance R-R. All the electrocardiograms changes caused by the action of the unconditioned stimulus were almost completely reproduced by the action of the conditioned stimulus, with slight differences in different dogs.

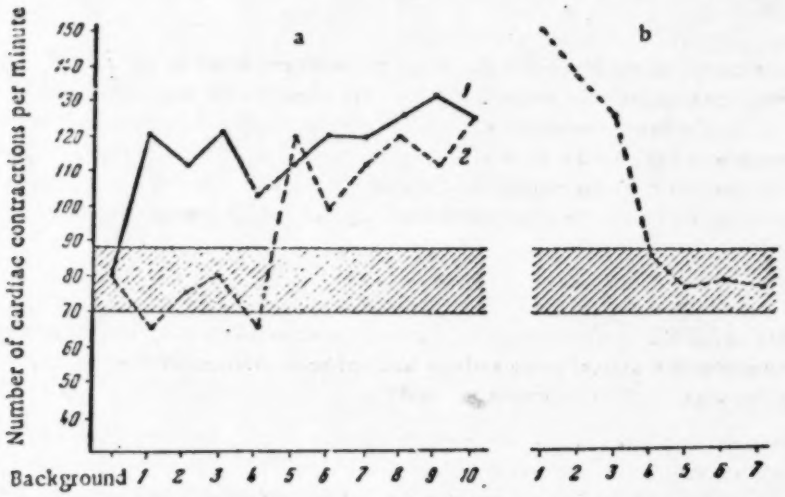

Fig. 1. Formation (a) and extinction (b) of proprioceptive conditioned reflex on heart in the dog Jack.
Along the horizontal – background and ordinal numbers of reinforcements or non-reinforcements of conditioned stimulus by unconditioned. Shaded strip – normal range of rhythm; 1) greatest rhythm changes under the influence of the physical load in the given experiment; 2) corresponding rhythm change caused by the action of the conditioned stimulus alone.

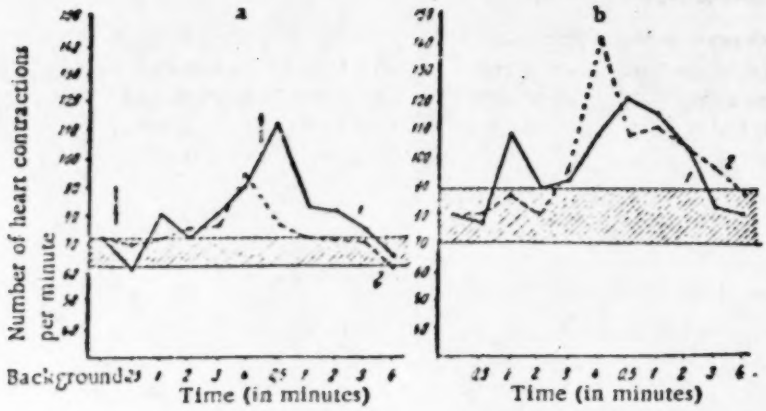

Fig. 2. Conditioned stimulus reproduction of the effect of the unconditioned stimulus. In the dog Treasure (a), in the dog Jack (b). Arrow down – beginning of stimulus action; Arrow up – cessation of stimulus action; 1) unconditioned reflex; 2) conditioned reflex.

The graphs included showing the rhythm changes in heart activity caused by the action of the unconditioned and conditioned stimuli were made from experiments on the dog Treasure after 20 combinations and on the dog Jack after 16 combinations of the stimuli (Fig. 2). They show that the conditioned stimulus effected exactly the same type of change in the heart activity as did the physical load. This applies to all the elements of the electrocardiogram. With the use of the static load, the maximal changes occurred during the period immediately after the action of the unconditioned stimulus had stopped, but, with the use of the conditioned stimulus, they occurred during the last minute of the stimulus action. However, this only characterized the given phase of the animal's manifestation of a reaction to the unconditioned and conditioned stimuli, and was not invariably observed.*

The fixed proprioceptive conditioned reflexes on the heart remained in the animals for a long time after the conclusion of the experiments. We twice interrupted the experiments after the fixation of the conditioned reflexes in two animals: the first interruption was 70 days for the dog Treasure and 85 days for the dog Jack; the second interruption was 135 days for both animals. Testing the effect of the conditioned stimulus after such interruptions showed that this stimulus caused the same changes in heart activity as before the experiments were interrupted. We must mention that the experimental set-up had a most marked effect on heart activity in these experiments. Noticeable changes in the animals' electrocardiograms made after the interruption were observed after the influence of the former experimental conditions alone, without the use of the positive conditioned stimulus. Consequently, the whole complex of the experimental conditions served as a conditioned stimulus to the animals, as well as the M120, even though reflexes to the surrounding had been eliminated before the work was begun, and changes in the animal heart activity had not been effected by the experimental set-up before the interruption of the work.

Although the fixed conditioned reflexes on the heart remained for several months after the dog's removal from the work, their active elimination was quickly accomplished. We found that it sufficed to place the animal a few times in the conditions of the earlier experiments and use the conditioned stimulus without reinforcement. In order to eliminate the proprioceptive conditioned reflexes, we put the animals on a bench for 15-20 minutes in the same room where the conditioned reflexes had been formed, and, once a day, used M120 without the reinforcement of the static load. After this had been repeated 3-4 times, the heart activity of the dog Jack returned to the original level under these conditions (see Fig. 1). The conditioned reflex was eliminated just as quickly in the other dogs.

The extinguished proprioceptive conditioned reflexes on the heart could be restored in the dogs after 1-2 combinations of the conditioned and unconditioned stimuli.

* See Sapov, I. A., Byull. Eksptl. Biol. i Med., 1957, Vol. 43, No. 1, p. 14.

An inhibitory conditioned reflex was formed in the animals after 1-4 uses of the differentiation stimulus. The first use of M60 on the dog Treasure retarded the rate of heart activity and prolonged the electric systole of the heart, as shown by the expansion of the complex QRS.

Figure 3 shows the changes in the rhythm of heart activity caused by the action of the positive and negative conditioned stimuli.

The second use of M60 (in the following experiment) caused no change in the animal's heart activity. Later, in all of the experiments, M60, used once, appeared firmly fixed as a differentiation. The third group of graphs (Fig. 3) shows the changes in heart activity occurring after many uses of the stimuli.

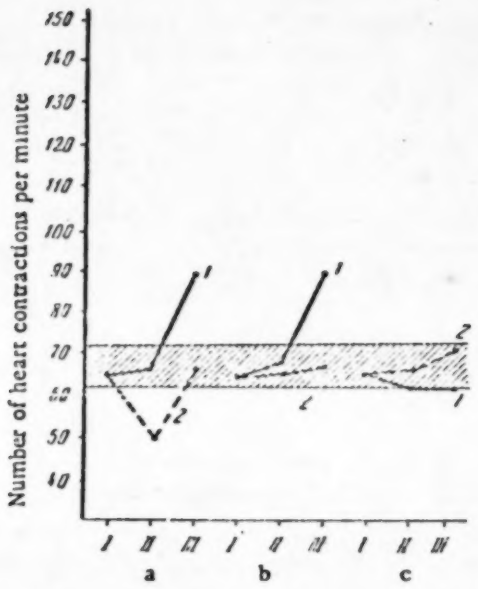

Fig. 3. Differentiation formation in the dog Treasure.
I) background; II) during the stimulus action; III) after the stimulus action. 1) positive conditioned stimulus (M120); 2) negative stimulus (M60). a) after one combination of M120 with the static load during the restoration of the conditioned reflex, the first use of M60; b) after 6 combinations of M120, the second use of M60; c) after 69 combinations of M120, the 15th use of M60.

These then were the results of some of the experiments conducted in order to examine certain properties of proprioceptive conditioned reflexes on the heart. They indicate the importance of influences from the proprioceptors in the adaptation of the heart activity to the ever changing conditions of natural existence and, possibly, the leading role played by proprioceptive influences in the regulation of heart activity.

Proprioceptive conditioned reflexes on the heart are quickly formed; the effect of the unconditioned stimulus is completely reproduced by the conditioned stimulus; these reflexes persist for a long time after the removal of the animal from the experimental surroundings, but can be quickly eliminated as well as quickly restored; a differentiation is formed after several uses of a nonreinforced stimulus – all these are properties of natural conditioned reflexes. The animal encounters daily many stimuli which invariably cause motor reactions, which, in turn, specify changes in the activity of the heart. A great many natural conditioned reflexes can be formed on the basis of unconditioned proprioceptive reflex effects on the heart. By the formation of one or two artificial proprioceptive reflexes, we add but little to the great quantity of natural proprioceptive conditioned reflexes. However, by this method, one can determine certain of their characteristics and, therefore, begin to understand the remarkably varied changes in heart activity which are observed both with the influence of stimuli directly causing a motor reaction and with the action on the body of all the environmental conditions under which the action of the stimuli occurs or occurred.

SUMMARY

Proprioceptive conditioned reflexes on the heart were studied in 5 dogs with the aid of electrocardiography. These reflexes are formed after 6-10 combinations of conditioned and unconditioned stimulations and are preserved for a long time in the intervals between experiments. Their frequency is actively increased, if not reinforced, 3-4 times and are restored after 1-2 combinations.

Differentiation takes place after 1-4 stimulations. These properties show the leading role of proprioceptive conditioned reflexes in adaptation of the heart activity to the changing conditions of the body.

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ON THE COORDINATED CHANGES IN THE BLOOD SUPPLY OF THE BRAIN AND EXTREMITY WHICH OCCUR DURING THE PROCESS OF FALLING ASLEEP

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Research on the circulation of such organs as the muscles and digestive glands shows that their blood supply clearly relates to their condition of activity.

The problem concerning the changes in the cerebral blood supply which are associated with the various degrees of brain activity is more complicated.

There are observations that, when the brain is active, its blood supply usually increases, for example, under the influence of light, sound or other stimuli [4]. Information on the blood supply of the brain during sleep is extremely contradictory. Along with data to the effect that both narcotic and natural sleep are attended by contraction of the cerebral vessels [11,14], there are also observations that the cerebral vessels dilate during sleep [6,18]. These diametrically opposed opinions can be explained by the fact the observations were done at different periods of sleep, on different subjects and, finally, with different methods.

Most of these methods – studying the circulation in the circle of Willis, plethysmography of the brain, observing the surface vessels of the brain through a trephined window – are meant for use in short experiments, and, therefore, the experimental study of this problem is difficult, until recently being limited to observations on people with a cranial defect [5].

EXPERIMENTAL METHODS

We had studied the changes occurring in the blood supply of the brain during natural sleep in a long-term experiment on a dog [12] by means of a plethysmographic method developed in our laboratory [1].

We fastened a special plug in a trephined window of the cranium with a branch tube for connection with a Marey's capsule. In a series of experiments, two plugs were simultaneously connected with the recording system and were placed diagonally at opposite poles of the cranium to allow for mutual compensation of the intracranial pressure with certain movements of the animal's head.

Since there is a close connection between the cerebral and the peripheral blood supply [3, 4, 18], we recorded a plethysmogram of an extremity at the same time, for which purpose we used a simple air plethysmograph, similar in form to the recently proposed water plethysmograph for an extremity [9].

The experiments were done on five dogs, Jack, Barbos, Gray, Ryzhik and Kakvas. All the dogs were trained to lie on a bench, and they easily fell asleep and after the removal of external stimulations.

EXPERIMENTAL RESULTS

The process of falling asleep was clearly shown by the kymogram (Fig. 1) in all of the experiments: the level of the brain plethysmogram rose, and the amplitude of the pulsations increased, while the level of the extremity plethysmogram, on the other hand, fell. The curve, moreover, became wavy. That these changes

were not connected with the movements of the dog is shown by the fact that the rod registering the movements of the head and extremity drew a horizontal line (Fig. 1).

A similar picture was observed in all of the experimental animals during medicamental sleep as well as natural sleep. The advent of morphine sleep was characterized by the same typical discrepancy between the cerebral and pheripheral plethysmograms and by an increase in their oscillation. However, these changes were temporary when effected by morphine, and were only observed at the beginning of the narcotic's action. In natural sleep, they occurred regularly each time the dog fell asleep after being repeatedly awakened.

The fact shown in these experiments that, simultaneously, the flow of blood to the brain is intensified while the peripheral blood supply is reduced can therefore be considered characteristic to the process of transition from the state of being awake to that of sleep.

Fig. 1. Coordinated changes in the blood supply of the brain and extremity during the process of falling asleep (in the dog Jack).
Curves from top to bottom show: respiratory movements, plethysmogram of brain, plethysmogram of extremity, movements of head, movements of extremity, indication of time (in 2 second marks).

In order to investigate the feasibility of such an "antagonistic" effect on the vessels of the brain and extremity from the standpoint of the vasomotor center, we conducted short experiments on dogs, stimulating the floor of the fourth ventricle at the places corresponding to the situation of the vasomotor center mechanisms [10]. We found that by stimulating certain portions of the floor of the fourth ventricle, one could reduce the blood supply of the extremity, while increasing the blood supply of the brain. Since the medulla oblongata was exposed when the cranium was opened, the conditions for plethysmography had sharply deteriorated. We therefore recorded the pressure in the circle of Willis in these experiments in order to determine the blood supply of the brain. The curve (Fig. 2) shows that the blood supply of the brain increased (rise of pressure in the circle of Willis), while the blood supply of the extremity decreased; arterial pressure in the animal changed slightly at the same time. This suggests the presence of a central nervous mechanism which redistributes the blood between the vessels of the brain and extremity in the way observed in our experimental dogs as they fell asleep.

The works of many authors have shown the great dependency of the cerebral blood supply on the concentration of carbon dioxide [13, 16, 17 and others]. The inhalation of air with a 10% content of carbon dioxide doubles the cerebral blood supply, while hyperventilation can reduce by half the natural flow of blood through the brain [15].

These and numerous other works concerning the dilatation of the cerebral vessels under the influence of

Fig. 2. Coordinated changes in the blood supply of the brain and extremity with stimulation of the floor of the fourth ventricle in a short experiment on a dog. The arrow shows the moment of stimulation with an induced current (distance of 230 mm between the induction coils). Curves from top to bottom show: respiratory movements, blood pressure in circle of Willis, plethysmogram of extremity, blood pressure in carotid artery.

carbon dioxide are particularly important in the light of the data obtained by A. A. Kedrov and A. L Naumenko [5] and their notion that carbon dioxide is physiologically important to the regulation of the tonicity of the cerebral vessels.

According to A. A. Kedrov and A. L Naumenko, "the intracranial neurovascular apparatus possesses a special reactivity to a surplus of carbon dioxide." At the same time, the amount of carbon dioxide in both the alveolar air and the blood is known to increase during the process of falling asleep.

This suggests that carbon dioxide activates the mechanism which, as we observed, increases the cerebral blood supply while the animal is falling asleep when the excitability of the brain stem is reduced [8, 12].

We conducted special experiments to test this effect of carbon dioxide. The head of a dog was enclosed in a transparent case made of thin plastic. Air containing 4-5% carbon dioxide was fed into the case from a 100-liter Douglas bag. The percent of carbon dioxide was controlled by a Golden gasometric analyzer. The plethysmograms of the brain and extremity recorded while the animal was in a tranquil state and under conditions of a surplus of carbon dioxide showed changes in the blood supply similar to those observed during the process of falling asleep. That carbon dioxide participates in the "sleep" redistribution of the blood is further indicated by the fact that respiratory changes (see Fig. 1) occurred almost simultaneously with the coordinated changes in the blood supply of the brain and extremity observed during the development of natural sleep.

SUMMARY

Long experiments were performed on 5 dogs. Changes in the blood supply of the brain and extremity during natural sleep and that induced by morphine were recorded by plethysmography. Coordinated changes were found in the blood supply of the brain and the extremity in the process of falling asleep, i.e., the blood supply of the brain was increased while the blood supply of the extremity reduced. It was demonstrated that such redistribution of the blood depended on the activity of the vasomotor center. Short experiments were performed on dogs with electric stimulation of the vasomotor center in the area of the fourth ventricle. Coordinated changes in the blood supply of the brain (increased pressure in the circle of Willis) and in the extremity (decrease of the plethysmogram) were recorded. Besides, data were obtained that the nervous mechanism of redistribution of the blood during sleep is influenced by carbon dioxide. In long experiments on dogs which were kept in the atmosphere, containing 4-5% of carbon dioxide, the same coordinated changes were found in the blood supply of the brain and extremity as during the process of falling asleep.

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DYNAMICS OF BIOLOGICAL NORMS IN MONKEYS

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It is desirable, for the solution of certain biological and medical problems, to use monkeys as experimental animals, since their anatomy and physiology are most closely approached to the human. A difficulty encountered in experimental work with such material is that there is a paucity of data concerning the biological norms of different species of monkeys.

It is generally known that many physiological and biochemical indexes vary according to the emotional state of the animal. Because of the extreme lability of nervous processes in monkeys, emotional excitation arising during an experiment may be so intense as to render the data recorded very different from physiological norms [1].

L. A. Utkin and M. L. Kuksova [4] have shown that systematic repetition of the procedure of blood sampling, day after day, under identical conditions, may lead to a marked fall in leucocyte count, as compared with the initial values. These authors consider that the nature of the change in leucocyte count is analogous to the extinction of a conditioned reflex. The fall in the leucocyte count observed after repeated sampling is regarded as being the result of an inhibitory process in the nerve centers responsible for the control of the composition of blood cells. The high leucocyte count observed initially is, in the view of these authors, due to a physiological leucocytosis, evoked by all the stimulating factors associated with the experimental conditions.

The present research was undertaken with the object of ascertaining whether similar effects of those encountered in determining leucocyte count are also found for other biological norms.

EXPERIMENTAL METHODS

The experimental material consisted of adolescent females of the following species: 3 Hamadryad mandrills, 2 Macacus rhesus, 3 marmosets, 1 capuchin, 1 gelada. The animals were taken for experiment twice weekly, before breakfast, on the same days and at the same times, and the manipulations were performed by the same experimenters, in the same order. The minute pulse rate and respiratory rate were measured, as well as the arterial pressure (Korotkov's method); blood samples were taken from the right median vein, and the rectal temperature was taken.

From 10 to 19 examinations were made of each animal. The apes remained in their usual living quarters on the days when they were not being examined.

The blood samples were oxalated (potassium oxalate); sugar was determined in one portion by the Hagedorn-Jensen method, and total cholesterol was determined for 6 apes by M. A. Levchenko's method.

EXPERIMENTAL RESULTS

Repeated blood sampling, under identical conditions, led in all cases to a fall in the blood sugar content (Table 1), and to a definite lowering of the blood cholesterol level (Table 2).

TABLE 1

Changes in Blood Sugar Levels in the Course of Repeated Sampling

Name of ape	Species	No. determin.	Duration observ. period, (days)	Blood sugar in mg %(mean values)	
				first 5 determin.	last 5 determin.
Pion	Mandrill	16	60	93	88
Zher	»	17	60	109	98
Azon	»	19	60	107	87
Artam	Rhesus	17	59	82	73
Slepen	»	18	56	88	83
Beglets	Marmoset	17	63	102	87
Bering	»	17	59	86	83
Erdzhis	»	18	56	107	81
Klaro	Capuchin	10	35	109	93
Kipr	Gelada	10	35	113	108

TABLE 2

Changes in the Blood Cholesterol Level of Apes During the Course of Repeated Sampling

Name of ape	No. determin.	Duration observ. per. (days)	Whole blood cholest., mg %			
			on 1st day	mean of first 5 determin.	on last day	mean of last 5 determin.
Zher	11	38	150	157	102	134
Azon	10	32	146	154	85	133
Artam	11	38	200	189	135	167
Slepen	10	32	207	212	152	201
Bering	11	38	167	173	122	152
Erdzhis	10	32	200	183	92	142

Table 3 presents analogous data for arterial pressure. In 9 cases out of 10 there was a definite tendency towards lowering of pulse pressure, due either to lowering of maximum, or raising of minimum, pressure, or to both factors acting simultaneously. The greatest final changes in pulse pressure were found for the apes Azon, Erdzhis, and Klaro.

In no case did we observe any significant rise in maximum blood pressure over the period of the observations, whereas definite falls in pressure were noted for individual animals. Thus the first readings taken from the mandrill Pion would qualify her for inclusion in the hypertensive group, whereas the last measurements of blood pressure, pulse rate, and respiratory rate fell within the normal range established by G. O. Magakyan [3].

A fall in pulse rate was observed after repeating the procedure under standard conditions in 7 apes.

No upward or downward tendencies were observed for the respiratory rate and the body temperature.

We conclude from our results that many physiological and biochemical indexes measured in the early stages of the observation period reflect the effects of all the stimuli associated with the experimental set-up, which give rise to pronounced orientational and defensive reflexes. The changes found in these indexes as the

TABLE 3

Changes in Blood Pressure of Apes During the Course of Repeated Measurements

Name of ape	No. measurements	Duration observ. per. (days)	Mean values blood pressure					
			of first 5 measure.			of last 5 measure.		
			maxi-mum	mini-mum	pulse	maxi-mum	mini-mun	pulse
Pion	15	56	157	91	66	121	72	49
Zher	11	38	129	62	67	132	64	68
Azon	11	35	162	42	120	159	72	87
Artam	11	39	157	78	79	162	96	66
Slepen	10	32	179	108	71	164	106	58
Beglets	17	63	149	84	65	140	87	53
Bering	11	38	165	93	67	151	100	51
Erdzhis	19	32	226	62	164	201	132	69
Klaro	17	63	156	39	117	156	91	65
Kipr	10	35	172	77	95	160	77	83

measurements were repeated may be ascribed to extinction of the emotional reactions caused by the experimental conditions.

It is possible that the indexes which did not undergo any marked changes during the observation period (body temperature, respiratory rate) are subject to different regulatory mechanisms, in view of their different physiological significance. This question requires further study. The possibility is not excluded that a more prolonged extinction period would have given more pronounced and uniform shifts of the values of all the indexes in all the experimental animals.

Although the experimental animals belonged to various genera and species, and although they were studied in two groups, at different seasons of the year, the systematic repetition of the measurements under standardized conditions gave identical shifts of a number of indexes. Our findings for the changes in leucocyte count a peripheral blood confirm those of L. A. Utkin and M. L. Kuksova [4].

SUMMARY

Various species and genera of monkeys were systematically examined in standard conditions. These repeated investigations were conducted for several weeks in succession. It was found that concentration of sugar and total cholesterin was decreased in the venous blood. These changes should be considered to be the result of decline of emotional shifts which were caused by experimental performance.

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PATHOLOGICAL PHYSIOLOGY AND GENERAL PATHOLOGY

THE USE OF OXYGENATED BLOOD IN EXPERIMENTAL RESUSCITATION

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There are references in the literature to the desirability of saturating with oxygen blood that is to be fed intra-arterially in order to restore the function of individual organs or the organism as a whole [1, 2, 8, 14]. It is known that when blood is administered intra-arterially it by-passes the lesser circulation and passes directly into the blood vessels of vitally important organs which are particularly sensitive to hypoxia, whereas on being administered intravenously the blood passes through the lungs where it is saturated with oxygen prior to entering the greater circulation. In connection with this, the question of oxygenating blood to be given intra-arterially has acquired considerable importance. In the practice of blood transfusion two methods of blood oxygenation are known and have been applied: bubbling of pure oxygen through the blood and addition to it of 3% or 30% solution of hydrogen peroxide. A number of authors [1, 2, 3, 4, 9, 11] saturated blood to be used intra-arterially with oxygen by adding 0.5-1.0 ml 3% or 30% solution of hydrogen peroxide and observed favorable results. However, in recent years, greater and greater attention has been directed to oxygenation of blood to be given intra-arterially by passing through it pure oxygen under pressure [6, 7, 10, 12, 13, 15]. Special apparatuses are in use for this purpose in which oxygenation of the blood takes place both before and during its administration. It must be noted that these reports are based on small numbers of observations, and the questions concerning the most effective means of oxygenating blood as well as of the effect of oxygen-saturated blood on the restoration of vital functions of the organism remained insufficiently clarified. It appeared interesting in this connection to discover the relative effectiveness of combined methods of resuscitation with intra-arterial administration of nonoxygenated or oxygenated (by various methods) blood as regards the process of restoration of the vital functions of animals following clinical death produced by exsanguination.

EXPERIMENTAL METHODS

Forty experiments were performed on dogs. Following a preliminary subcutaneous injection of 2% solution of pantopon (8 mg per 1 kg body weight) the femoral vessels were dissected bilaterally under ether anesthesia. The left femoral artery was connected by a system of rubber tubes to a mercury manometer for recording of arterial blood pressure. Respiration was recorded by means of a cuff placed round the animal's thorax and connected to Marey's tambour. Arterial pressure and respiration were recorded on a kymograph. Exsanguination by way of the right femoral artery was performed after preliminary administration of heparin. The amount of blood drained consituted 62%, on the average, of the total volume of blood if it is taken that the total amount of blood equals 9.2% of the total body weight. Restoration of vital functions began 5-7 minutes after onset of clinical death. The technique has been developed by Professor V. A. Negovsky and his collaborators. The method consists essentially of centripetal feeding of blood into the artery and simultaneous application of artificial respiration by means of apparatus which blows air into the lungs. Oxygenation of the blood was effected in 17 experiments by addition of 2.0 ml 3% solution of hydrogen peroxide per 300-400 ml blood. Hydrogen peroxide was placed in the bottom of the flask together with glucose (0.5 g 40% solution per 1 kg body weight), the flask was then filled with blood, with shaking. The blood usually acquired a bright color during this procedure. In 8 experiments the blood was oxygenated by means of a jet of oxygen from a cylinder, under pressure

of 250 mm Hg. At the moment of intral-arterial introduction the escape of oxygen from the flask was stopped and a positive pressure (which could be regulated) of oxygen on blood was created; the blood entered the artery under this pressure. In a number of experiments the degree of oxygen saturation of arterial blood was determined, using the Kipp hemoreflector, at different stages of dying and resuscitation. The same method was used to determine the oxygen saturation of the blood drained from the animal before and after oxygenation of this blood.

EXPERIMENTAL RESULTS

Three series of experiments were carried out. In the first series (17 experiments oxygenation of the blood to be administered was effected by the addition of 2.0 ml 3% solution of hydrogen peroxide (freshly prepared) per 300-400 ml of blood. This led to an increase in oxygen saturation of blood in the flask from 85-95% to 90-98%. The period of dying in most animals of this series (9 of 17) lasted from 6 minutes 24 seconds to 9 minutes 48 seconds, in 5 animals this period was of medium duration – from 10 minutes 10 seconds to 14 minutes 35 seconds – in 2 animals it was prolonged – 21 minutes 28 seconds and 22 minutes 48 seconds. Duration of clinical death in the animals in 10 experiments was from 4 minutes 54 seconds to 5 minutes 10 seconds and in 7 experiments – from 7 to 8 minutes.

Cardiac activity was restored in most experiments (6 of 10) after 5 minutes of clinical death 20-27 seconds following the beginning of resuscitation, in 3 experiments following 32-35 seconds and in one experiment (Experiment 1) following 12 seconds. In the majority of experiments (5 out of 7) cardiac activity was restored after 30-34 seconds in animals which had sustained clinical death lasting 7 minutes, and 2 experiments it was restored 42 and 54 seconds after beginning resuscitation.

Restoration of respiration in animals which had sustained clinical death lasting 5 minutes occurred after 1 minute 36 seconds – 2 minutes 56 seconds from the beginning of resuscitation. Experiments 6 and 7 were exceptional in that the period of dying was prolonged (14 minutes 35 seconds and 22 minutes 48 seconds) and respiration in these animals was restored after 4 minutes 33 seconds and 7 minutes from the beginning of resuscitation. Restoration of corneal reflexes in most animals after 5 minutes of clinical death occurred after 10 minutes 40 seconds – 16 minutes, in 2 experiments after 7 minutes 24 seconds and 7 minutes 20 seconds and in one experiment in which restoration of respiration was delayed the corneal reflexes appeared 20 minutes 12 seconds after the beginning of resuscitation.

Spontaneous respiration in animals which had sustained clinical death lasting 7 minutes was resumed 1 minutes 42 seconds – 4 minutes 54 seconds from the beginning of resuscitation. In one experiment the animal's respiration was restored after 6 minutes 40 seconds; this was connected with hyperventilation and damage of pulmonary tissue during artificial respiration. The corneal reflexes in most animals appeared 15 minutes 6 seconds – 23 minutes 36 seconds, in 2 experiments 10 minutes 12 seconds and 9 minutes 6 seconds after the beginning of resuscitation.

Restoration of central nervous system function occurred in 8 out of 10 animals subjected to clinical death lasting 5 minutes. Complete restoration of vital functions in most of the animals in this group occurred 36-60 hours after the experiment. Complete recovery of the animals was judged only by their outward behavior. No conditioned reflex studies of higher nervous activity were carried out. Of the 7 animals subjected to 7-minute clinical death 2 died soon after the experiment while 5 survived; in 4 of these central nervous system function recovered but in one the recovery was partial, since neither vision nor hearing was restored.

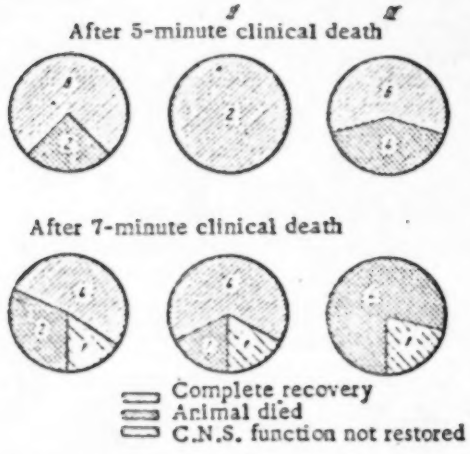
In the second series (8 experiments) oxygenation of the blood to be administered intra-arterially was effected by passing through it a jet of oxygen from a cylinder under pressure (250 mm Hg) at the rate of 600 ml/min. Oxygenation of the blood was started 10-15 minutes before resuscitation, the oxygen saturation of the blood rising during this period from 82-94 to 96-97%. The blood was fed into the artery under the pressure of oxygen. The period of dying in this series varied from 8 minutes 10 seconds to 16 minutes 52 seconds. Clinical death lasted 5 minutes 21 seconds and 5 minutes 3 seconds in 2 experiments and from 7 to 7 minutes 5 seconds in the remaining 6 experiments. Cardiac activity was restored in most of the animals in this series 45 seconds – 1 minute 15 seconds and in one animal 35 seconds after the beginning of resuscitation. During the recovery of cardiac activity one animal developed fibrillation which was abolished by a condenser discharge, the cardiac activity being again restored 3 minutes 55 seconds from the beginning of resuscitation. The somewhat later restoration of cardiac activity in these experiments as compared with those of the previous series may be explained by the fact that the pressure necessary for administering the blood from the

flask developed over a period of 40-50 seconds and during the initial stage of resuscitation the blood entered the artery under insufficient pressure. As has been shown by the work carried out in the laboratory (V. A. Negovsky, E. M. Smirenskaya) it is impossible to create the necessary level of circulation in the coronary vessels supplying the heart muscle when the pressure is insufficient.

Respiration was restored in 4 animals after 3 minutes 15 seconds – 4 minutes 18 seconds from the beginning of resuscitation. In 3 animals respirations appeared after 6 minutes 2 seconds – 6 minutes 35 seconds and in one animal after 2 minutes 20 seconds. Corneal reflexes were restored in the majority of animals 13 minutes 5 seconds – 16 minutes 45 seconds after the beginning of resuscitation, in 2 animals – after 18 minutes 35 seconds and 20 minutes 5 seconds. Subsequently, of the 8 animals in this series 7 survived and one died 24 hours after the experiment; complete recovery of functions occurred in 6 dogs, in 4 of these after 2-3 days, in 2 after 5 and 10 days following the experiment. Only partial recovery of central nervous system function took place in one animal: hearing and vision were not restored 2 weeks after the experiment.

The third series consisted of 15 control experiments in which nonoxygenated blood was used. In 10 experiments the duration of clinical death ranged from 4 minutes 54 seconds to 5 minutes 10 seconds, the period of dying lasting from 8 minutes 5 seconds to 17 minutes 55 seconds; in 5 experiments clinical death lasted 6 minutes 59 seconds – 7 minutes 9 seconds and the period of dying ranged from 6 minutes 6 seconds to 12 minutes 6 seconds. The amount of blood loss varied from 51 to 81% of the total amount of blood.

Cardiac activity in the animals whose clinical death lasted 5 minutes was restored after 27-42 seconds. Only in one experiment it was restored 1 minute 12 seconds from the beginning of resuscitation. Animals which had sustained 7-minute clinical death showed restoration of cardiac activity after 38-55 seconds. Respiration in the former group of animals was restored after 2 minutes 35 seconds – 3 minutes 50 seconds and in two animals after 7 minutes 16 seconds and 5 minutes 54 seconds from the beginning of resuscitation. In the case of the latter group, respiration was restored after 3 minutes 54 seconds – 8 minutes 30 seconds from the beginning of resuscitation. Corneal reflexes in animals whose clinical death lasted 5 minutes appeared after 12 minutes 20 seconds – 21 minutes 10 seconds and in one animal after 8 minutes 20 seconds. Of the 10 animals which sustained 5-minute clinical death 6 survived; of the 5 animals subjected to 7-minute clinical death one survived, but its vision failed to recover. The remaining animals died in the course of the first twenty-four hours after the experiment.



Combined method of resuscitation of animals. I) Use of blood oxygenated by addition of 3% hydrogen peroxide solution; II) use of blood oxygenated by passage of a jet of oxygen under pressure; III) use of nonoxygenated blood.

It is well known that the time of restoration of cardiac activity, respiration and corneal reflexes are of great significance in the process of animal resuscitation. But the most important indication of the effectiveness of combined methods of resuscitation with intra-arterial administration of oxygenated or nonoxygenated blood is the restoration of C.N.S. function in the animal. Comparison of experimental results by stages of resuscitation reveals that animals resuscitated after 5 and 7-minute clinical death by intra-arterial administration of oxygenated blood show a tendency to accelerated restoration of cardiac activity, respiration and corneal reflexes. Thus, 9 out of 17 animals in the first series showed definite acceleration of recovery of cardiac activity as compared with control animals which sustained analogous periods of dying and clinical death. In 7 animals of the first series more rapid restoration of respiration and, in a number of experiments, of corneal reflexes were observed. The advantage of using oxygenated blood is however, most vividly demonstrated by a comparison of the results obtained in the 1st and 2nd series with those of the control experiments. As can be seen from the figure, the most favorable results were obtained in the first and second series of experiments, in which oxygenated blood was used. Of 12 animals which sustained 5-minute

clinical death and were resuscitated by oxygenated blood, C.N.S. function was restored in 10 dogs, whereas of 10 control animals with analogous periods of dying and of clinical death complete recovery of function only occurred in 6 dogs. Of 13 animals which sustained 7-minute clinical death and were resuscitated by intra-arterial administration of oxygenated blood 10 survived, 8 with recovery of C.N.S. function, while of 5 control animals partial restoration of function was noted in only one dog. It must be mentioned that in the experiments of the second series complete restoration of vital functions occurred in the majority of animals (6 out of 8), despite delayed restoration of cardiac activity, and in some experiments of respiration as well, as the result of faulty technique.

The mechanism of the favorable effect of oxygenated blood on restoration of vital functions revealed in these experiments is not yet sufficiently clear. Although it may seem that the difference in the degree of oxygen saturation of the blood before and after its oxygenation is relatively small, in practice it exerted a definite effect on the physiologic results of the experiments. Apparently, the additional oxygenation of the blood, even though it did not result in a great difference in the level of saturation, exerted a significant effect on the increase of partial pressure of gaseous oxygen in the blood entering the artery. This would assist a somewhat more vigorous course of oxidative processes in the tissues which undoubtedly constitutes a positive factor in the course of subsequent restoration of the animal's vital functions. It must be pointed out that the favorable effect of increasing the partial pressure of oxygen on the restoration of cardiac activity has also been noted by V. S. Shapot and G. M. Pruss [5] and by some other authors.

The present experiments have thus demonstrated the positive effect produced by oxygenated blood on the process of restoration of the organism's vital functions. This allows the hypothesis that clinical use of oxygenated blood for intra-arterial administration may also be beneficial in the treatment of terminal conditions.

SUMMARY

The effect of blood (oxygenated by various methods) on the process of recovery of vital functions of the organism following clinical death caused by loss of blood was studied. It was established in experiments on dogs that the percentage of survival with complete recovery of the vital functions was much greater in animals which were treated by oxygenated blood in comparison with those which received ordinary blood.

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ON THE REFLEX MECHANISM OF DISTURBANCES IN RESPIRATORY MOTIONS WITH LESIONS OF THE UPPER RESPIRATORY TRACT (IN THE PHENOMENON OF "SIMULATED ASPHYXIA")

ELECTROMYOGRAPHIC STUDY

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Studying the reflex mechanisms of respiratory disorders in lesions of the lungs and upper respiratory tract, we, like many other researchers [7], have often observed the complete conformity of the nose and diaphragm respiratory motions in tracheotomized animals.

We called these respiratory motions of the facial muscles in tracheotomized animals "simulated respiration" [8], as the phenomenon is similar to the "simulated feeding" of esophagotomized animals. "Simulated feeding" greatly affects the function of the gastro-intestinal tract, and "simulated respiration" has a similar effect on the act of respiration. If the nostrils of a tracheotomized animal are pinched, the animal's breathing will become slower, even though there is nothing to hinder the intake of air through the trachea. We named this phenomenon "simulated asphyxia." Klod Bernar [1] has already shown that constriction of the larynx causes similar respiratory changes.

From these observations, it follows that the inhibition of respiration up to prolonged pauses which occurs from the very first moments of mechanical asphyxia is actually of reflex origin.

In the experiments described above, we noticed the following fact. When simulated asphyxia was caused by pinching the laryx of a tracheotomized animal, the respiratory motions of the nose and thorax muscles differed considerably as to force, although they became equally retarded. The amplitude of the thorax motions remained about the same or even decreased, while the motions of the nose involved the greatest possible exertion of the muscles.

The difference in the reactions of the different groups of respiratory muscles in the phenomenon mentioned is important to the study of the reflex mechanisms of true asphyxia, and therefore these observations were made the subject of special investigations. We used the electromyographical method for this purpose, as by that method one can record the activity of separate muscle groups.

In this work, we made comparative studies of the electric activity of two respiratory muscle groups – the muscles of the alae nasi and the main respiratory muscle of the diaphragm which principally determines the volume of respiratory movements.

EXPERIMENTAL METHODS

The experiments were done on 14 rabbits weighing 2-3 kg each. The animals were fixed on their backs and not anesthetized. We recorded the respiratory movements of the nose and diaphragm with an amplifier and with a Scleife oscillograph manufactured in the precision tool shop of the Lensoviet Leningrad Technological Institute. The usual amplification was used, with which the calibration impulse amplitude was 10 mm with a voltage of 1 mv.

Electrodes from a thin, steel needle covered with an insulating layer of shellac (except for the last 6-8 mm at the tip of the needle) were inserted into the muscular part of the diaphragm through the abdominal or thoracic wall (from the right) at least 1 cm apart. The entry of the needle was controlled according to the appearance of rhythmic group discharges, synchronized to the fluctuations of a pneumograph (the fluctuations of a ray from a small mirror attached to a Marey's capsule were recorded at the same time as the biocurrents of the respiratory muscles). The electrodes were fixed by "piercing" the diaphragm, i.e., by pricking it repeatedly near the place where the needle was inserted.

The nose or larynx was constricted with pincers wrapped in cotton or by the experimenter's hand in a rubber glove (the latter method was sufficiently effective and less traumatic) until the walls of the nostril or larynx were pressed together. In most of the experiments with nose constriction, a definite reaction could be obtained by pinching only one nostril. This enabled us to use the other nostril to record electromyograms of the nose muscles. A second pair of needles was inserted into the nose muscles, into the upper and lower portions of the right nostril – the sections expanding most with inhalation (the largest muscles are the m. levator alae nasi and the m. orbicularis oris).

EXPERIMENTAL RESULTS

The electric activity of the nose muscles with calm respiration was characterized by continuous impulsation, the rate of the predominant rhythm being about 150-200 impulses per second, according to the maintenance of constant tonicity of the open nostrils. The average discharge amplitude ranged from 0.3-0.5 to 2 mv, increasing with inhalation.

There was a greater increase in the discharge amplitude of the diaphragm during inhalation, although continuous activity is maintained between inspirations in the rabbit [13, 15, 16, 18, 20].

In true asphyxia, the electric activity of the alar nose muscles and the diaphragm muscles is considerably intensified. As soon as the reflexogenic zone of the upper respiratory tract is contacted, ordinarily, a discharge of high-amplitude impulses arises in the muscles of both the nose and diaphragm. This reaction lasts $\frac{1}{2}$-1 second and is nonspecific, since it is also observed with pressure on any other part of the animal's body. Typical respiration retardation occurs immediately after it, and the amplitude of the nose muscle action currents with inspiration increases sharply (to 4.5-5 mv, sometimes more). There are more sharply defined pauses with expiration, sometimes a complete cessation of activity. Then the same type of high-amplitude discharges also appear with expiration, corresponding to the expiratory efforts of the alar nose muscles.

In mechanical asphyxia, the changes in the electric activity of the diaphragm (as already described by Dittler [13], Wachholder, McKinley [20], Gesell [14] and others) completely correspond to the changes in the electric activity of the nose muscles (Fig. 1).

The same conformity has been observed in the case where asphyxia is caused by closing the opening of the tracheal tube, i.e., with direct stimulation of the upper respiratory tract reflexogenic zone excluded. The electric activity of the diaphragm does not change immediately, but gradually, over a period of 3-5 seconds, [14] as does the electric activity of the nose muscles.

With simulated asphyxia, the electric activity of the diaphragm and nose muscles differed from that observed with true asphyxia. Although the impulsation from the alae nasi retained signs typical of true asphyxia (sharp increase in amplitude – up to 5 mv – more definite intervals between inspiration and exspiration discharges), the impulsation from the diaphragm was completely different.

The inspiration discharges of the diaphragm, maintaining their gradually increasing character, became longer and longer and slower and slower; their amplitude usually remained about the same, increasing not more than $1\frac{1}{2}$-2 times if at all, never even approaching the sharp increase in amplitude characteristic of the nose

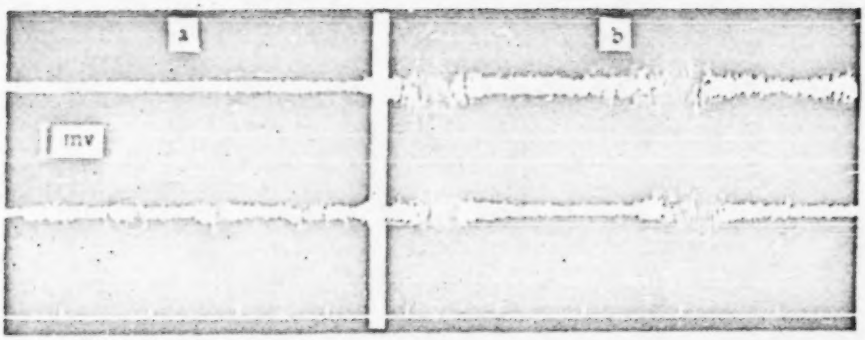

Fig. 1. Electromyograms of the respiratory muscles in true asphyxia.
Curves from top to bottom show: action currents of nose muscles, action currents of diaphragm, indication of time (in one second marks): a) original background; b) two seconds after constriction of the larynx – retarded respiration, simultaneous increase in discharge amplitudes of diaphragm and nose muscles.

muscles. We also noted that the amplitude changes with inspiration and expiration were less distinct (Fig. 2), again in contrast to the nose muscles.

Now why did the action currents of the nose increase in amplitude with simulated asphyxia, while those of the diaphragm did not? It would seem that impulsation from the respiratory center, changing due to the effect of extraordinary afferent stimulation from the constricted larynx or nose, would affect the constrictions of both the diaphragm facial muscles equally.

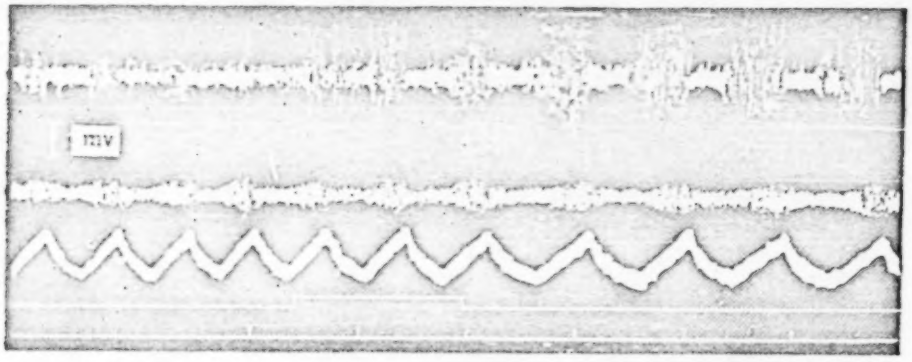

Fig. 2. Electromyograms of respiratory muscles in simulated asphyxia caused by closing one nostril of a tracheotomized animal.
Curves from top to bottom show: action currents of nose muscles, action currents of diaphragm muscles, pneumogram, indication of stimulation (closing nostril), indication of time (in 1 second marks). Respiration retarded as in true asphyxia, but character of changes in discharge amplitude is different – action current discharges of nose muscles are greater, those of the diaphragm muscles – smaller.

The mechanism of this phenomenon seems to be associated with peculiarities in the nervous regulation of the respiratory muscles. The impulses from the respiration center, before reaching the diaphragm and nose muscles, enter their motoneurons, especially the nuclei of the facial nerve (in the brain stem) and the nuclei of the phrenic nerve in the cervical part of the spinal cord (C_4).

The reaction of these motoneurons to impulses from the respiration center depends on their excitability, which is regulated by afferent influences from the periphery. These latter are different in true and simulated asphyxia.

In true asphyxia, both the motoneurons of the upper respiratory tract muscles and motoneurons of the diaphragm undergo sharp afferentation disturbances: in the first, this is due to the direct stimulation of the upper respiratory tract receptors caused by pressure on them, in the second, to the heightening of intrapulmonic pressure. This last factor is lacking in simulated asphyxia since, in tracheotomized animals, the lungs have free access to the outside air through the opening in the trachea.

As a result, the excitability of the phrenic nerve center is lower than that of the facial nerve center, which causes the impulsation from the respiration center to be manifested especially strongly in the nose muscles, and sometimes earlier than in the diaphragm.

Therefore, it follows that in true asphyxia, the intensified operation of the diaphragm is largely caused by the heightened excitability of its spinal center which, in turn, depends on the local, peripheral influences caused by extraordinary impulsation from the pleura, lungs and actual diaphragm.

The excitability of the respiratory muscle montoneurons can be raised by the influence of impulsation from the brain stem centers regulating muscular tonicity, as well as from the periphery due to impulsation from the receptors of the respiratory tract. In this way, serious respiratory disturbances, associated with a sharp increase in muscular tonicity, are known to occur both clinically [2, 9] and experimentally [3, 4, 10, 11, 12, 17] in decerebrate rigidity. These disturbances are especially pronounced during attacks of raised muscular tonicity [6]. Mislavsky [5] has already shown that these disturbances depend on disturbed montoneuron excitability, and not on disturbances in the function of the respiration center in the medulla oblongata. It has recently been established that these respiratory disorders disappear when the stem is transected at the level of the VIII pair of cranial nerves (below Deiter's nucleus), i.e., after a transection which also removes the phenomenon of decerebrate rigidity [10, 12, 19].

Therefore, both our experiments and the literature data show that, when analyzing respiratory disturbances, one must consider the condition of the peripheral neurons as well as that of the respiration center. The qualitative and quantitative characteristics of the respiration type in various pathological processes can evidently depend on the excitability of these motoneurons.

SUMMARY

Electric activity of the muscles of the nose and of the diaphragm was investigated in rabbits. It was revealed that the amplitudes of action currents of these muscles grow simultaneously in true asphyxia. In "simulated asphyxia," i.e., in disturbances of respiration caused by obstruction of nostrils or trachea in tracheotomized animals, the electric activity of the diaphragm is changed much less than in that of nose muscles. Analysis of this phenomenon suggests that the character of the movements of respiratory muscles is determined not only by the character of impulses from the respiratory center, but also by the change in excitability of peripheral motoneurons.

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SHORTENING OF REFLEX APNEA WITH EXPERIMENTALLY INDUCED HEART DEFECT

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Earlier conducted inveatigations have shown that changes in the functional condition of the centers regulating cardiac activity develop during compensated experimental aortic stenosis in rabbits. These changes are essential to the maintenance of continuous, compensating cardiac hyperfunction [1] as they are expressed by an increase in the reflexes intensifying cardiac activity, which are realized through sympathetic innervation, and by a decrease to 1/4-1/5 of the original level of the vagus reflexes inhibiting cardiac activity. Taking into account the close intercommunication of the circulation and respiration functions and especially the data indicating the reflex effect on the respiration center from the cardiac and vascular interoceptors [2, 3, 4, 5, 6, 8], one can propose that changes develop in the nervous regulation of respiration as well as in cardiac activity in experimentally induced heart defect. To prove this proposition, in this work we studied the effect of experimental aortic stenosis on respiration and on the development of unconditioned reflex apnea in animals.

EXPERIMENTAL METHODS

The experiments were done on 16 male rabbits weighing 2-2.5 kg. Aortic stenosis was induced by an earlier described method [1]. The characteristic functional, morphological and biochemical changes resulted from the constriction of the aortic orifice to 1/4-1/6 of its normal size by means of a lateral ligature. Respiration was electromyographically recorded; a cuff attached to a Marey's capsule was placed on the region of the chest and abdomen. The animal was fixed in position, lying on its stomach. Reflex apnea was induced by stimulating the upper respiratory tract with ammonia fumes. This was done by blowing air from a balloon through a double-necked jar, on the bottom of which was 100 ml of 25% ammonium hydroxide, into the rabbit's nostrils through a small tube, 3 mm in diameter. The receptors of the trigeminal nerve were stimulated, and this stimulation spread through the trigeminal centers to the respiration center, causing apnea. The duration of the apnea was measured in seconds on kymograms. We first conducted comparative experiments, comparing the duration of apnea in normal rabbits with that in 5 animals of the same weight and sex in which a heart defect had been experimentally induced one month before. Then dynamic experiments were done on 5 rabbits; the duration of the apnea was determined before the heart defect was developed and one month after its development. Three rabbits were used as a control; they were given an operation similar to that which was used to induce the aortic stenosis but the ligature on the aorta was not tightened, and therefore did not constrict the lumen. The animals were observed every other day; in each experiment, the stimulant was used 4-6 times at inervals of 2-3 minutes. The duration of apnea in each animal was determined a total of 110-140 times.

In part of the experiments recording the respiration during reflex apnea, the changes in oxygen saturation of arterial blood were observed by means of a Krebs oxyhemometer, with the photoelement placed on the rabbit's ear. The readings of the apparatus were recorded every 5 seconds for 3 minutes before the use of the ammonia fumes and for 5 minutes after their use. Oxyhemometry, when used without direct determination of blood hemoglobin saturation with oxygen according to Van Slyke, is known to be inadequate to establish the true oxygen

content in the blood. However, it does give a good idea of how this index fluctuates, counting off from a representative original level. We used 96% as the original oxygen content of the hemoglobin in these experiments. During reflex apnea, this index decreased to 92-88%, and then gradually returned to normal.

EXPERIMENTAL RESULTS

There was considerable variation among the different animals as to how long the apnea lasted, but apnea duration was more constant in the same animal, varying by 2-3 seconds from experiment to experiment. The Table gives the average apnea duration in 3 normal animals and in 5 rabbits with a heart defect, computed on a basis of 100 determinations per animal.

Duration of Reflex Apnea

Rabbit with heart defect		Normal rabbits	
No. of animal	Apnea (in sec.)	No. of animal	Apnea (in sec.)
1	9	11	16.5
2	7.5	12	15.5
3	12.5	13	21
4	7.5	14	18
5	10.5	15	16.5

The Table shows that the reflex apnea lasted from 15½ to 21 seconds in the normal animals and from 7½ to 12½ seconds in the animals with the compensated defect. Therefore, apnea duration in the animals with the defect was approximately half as long as in the normal animals.

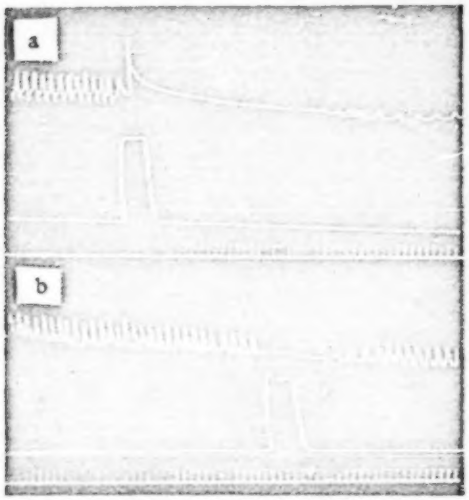

Fig. 1. Reflex apnea in rabbit No. 6 before creation of heart defect (a) and 1½ months after creation of heart defect (b). Curves from top to bottom show: respiration, indicated of stimulation, indication of time (in 1 second marks).

A month after the defect had been developed in all of the animals, dynamic experiments also showed a considerable shortening of the reflex apnea. The pneumograms of rabbit No. 6 made before the creation of the defect (Fig. 1, a) and 1½ months afterwards (Fig. 1, b) show that apnea duratinn in this animal was almost three times as short in the second pneumogram. In evaluating the fact of reflex apnea shortening, one should remember that the surplus of CO_2, which appears during the apnea, acts reflexively through the chemoreceptors and directly on the nerve cells to cause rhythmic activity in the given center; the apnea is shortened, and normal respiration is restored. In a given animal, the faster the concentration of carbon dioxide reaches the threshold level and the greater the excitability of the respiration center, the shorter reflex apnea will be, other conditions being equal. Therefore, the fact that the reflex apnea observed in the rabbits with the experimental heart defect was 2-3 times shorter must be due to either increased excitability of the respiration center, or the fact that the minute volume of the blood is decreased in aortic stenosis, while the CO_2 content of the blood increases faster than normally. However, the latter explanation contradicts the known fact that, in the animals with heart defect, the shortened apnea was attended by slighter and shorter decline in the O_2 content (Fig. 2). Moreover, even short apnea will probably cause the O_2 content of the blood to decrease sharply when the minute volume is decreased and the arteriovenous difference of O_2 saturation correspondingly increased. According to the experimental data of C. Wiggers [9], there is no

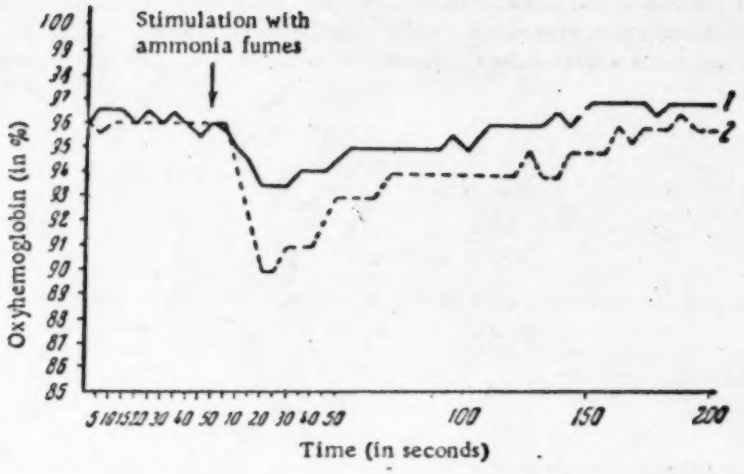

Fig. 2. Decrease of oxyhemoglobin content in blood during reflex apnea in rabbit with experimentally induced heart defect (1) and in the same animal before the creation of the defect (2).

decrease in the minute volume even when the aorta, where it leaves the heart, is 60-70% smaller in diameter, i.e., when the cross-section of the aorta is 8-9 times smaller than normally. Goldberg, Bacst and Baily [7], conducting a clinical physiological study on 26 patients with clear aortic stenosis, showed that the minute volume, the stroke volume and the arteriovenous difference in the oxygen saturation of the blood remain normal even when the degree of stenosis is considerable. These data allow the proposition that constricting the aortic cross-section by 4-6 times, as we did in our animals, i.e., by less than in Wigger's experiments, did not cause the minute volume to decrease either. Consequently, the shortening of the reflex apnea is due to the increased excitability of the respiration center.

SUMMARY

A shortening of the reflex apnea is observed in rabbits with compensated experimentally induced aortic stenosis in stimulation of the upper respiratory tract by ammonia.

Evidence obtained from oxyhemometric investigation gives base for conclusion that this phenomenon is the result of increased excitability of the respiratory center.

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CERTAIN CONSEQUENCES OF DESTROYING THE CAUDATE NUCLEI IN RABBITS AND DOGS

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One of the main tasks of physiology is to investigate the role of the basal ganglia. There is a mass of literature on this subject. Scientists are especially interested in the function of the striate body [1-7 and others], and particularly, in the function of one of its nuclei (nucleus caudatus).

In recent years, a series of authors have discovered new facts indicating that the caudate nuclei evidently send inhibitory and stimulating impulses to the cerebral cortex [8, 9, 10]. There are indications in the literature that the caudate nuclei are related to conditioned reflex activity [11, 12]. Nevertheless, the physiological role played by the separate nuclei of the striate body has not yet been clearly established. This prompted us to investigate the functional importance of the caudate bodies. For this purpose, we chemically caused unilateral and bilateral destruction of the caudate nuclei in rabbits and dogs and examined the consequences of our intervention.

EXPERIMENTAL METHODS

In our experiments, we first used an injection of alcohol, to which colophony was added to increase the viscosity, to destroy the subcortical nuclei. However, even the addition of the colophony did not completely prevent the alcohol from spreading and diffusing through the brain matter, which could cause an undesirable stimulating effect. Therefore, we used melted paraffin for this purpose in later experiments. With the localized destruction of the subcortical nuclei by such a method, conditions were created which were somewhat analogous to those attending a brain tumor (as, for example, pressure on the adjoining tissue).

Paraffin, at a temperature of 80-100°, was taken up into a hypodermic and injected in small amounts into the region of the caudate bodies (about 0.03 ml was injected into the rabbits, from 0.05 to 0.2 ml into the dogs). In the dogs, the injection was done through a puncture in the cerebral cortex at the level of girus cruciatus posterior. After this method had been developed in short experiments, we went on to study the consequences of destroying the caudate nuclei in long experiments. The operation was done on the dogs under morphine-ether-chloroform anesthesia, in the rabbits, under a local novocain anesthetic. The animals were observed for different periods of time (from 2 weeks to 3-6 months), depending on the conditions of the experiment, and then killed; their brains were examined pathoanatomically and, in some cases, histologically.

EXPERIMENTAL RESULTS

Experiments on rabbits. The caudate nuclei were unilaterally destroyed in 6 rabbits, bilaterally, in 7 rabbits. A control operation was done on three rabbits (cranial trepanation and two symmetrical punctures of the cerebral cortex). A total of 16 animals were observed. After the operation (Fig. 1), in the rabbits with bilaterally destroyed caudate nuclei, one could detect slight locomotor disturbances manifested by the "falling to pieces" of the paws in a jump and rather clumsy movements, which, 1-2 weeks after the operation could only be observed when the rabbits were on a slippery floor. A certain decrease in the tonicity of the leg muscles

was observed in the animals. The thresholds of the flexor reflex to an induced current were raised, but only during the first few days after the operation. There were no definite changes in the respiration and arterial blood pressure. Certain peculiarities in the general behavior of the animals appeared after bilateral destruction of the caudate nuclei. During the first days after the operation, the orientation reaction to the environment either became very weak or disappeared entirely in the rabbits. They sat passively in one place; if they were moved from this position, the animals often dashed off wildly (especially when tactile stimulation was employed) and hid in a corner.

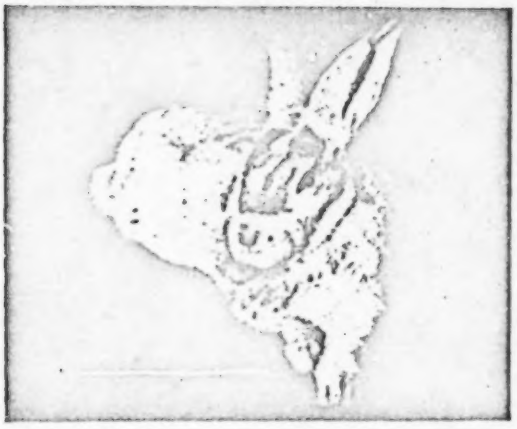

Fig. 1. Rabbit with caudate nucleus on right side destroyed shown the 5th day after the operation.

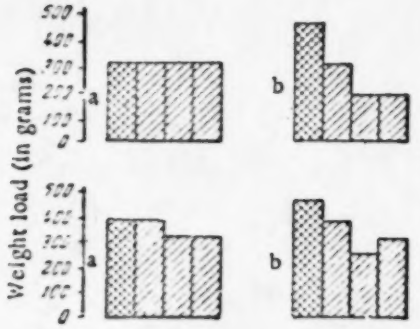

Fig. 2. Change in extensor tonicity of the muscles in Ace after destruction of the caudate nucleus on the left side.
I) anterior paws; II) posterior paws; a) left; b) right. Crosshatch) measurement before operation; slanting lines) measurement after operation; on the day of the operation and 7 and 14 days after it.

After unilateral destruction of the caudate nuclei, we often observed asymmetrical positions of the rabbits' head and body (bent towards injured side). Their movements were selectively orientated towards the injured side. On the opposite side of the body, the paws (especially the anterior one) were often in a semi-extended position, although the rabbits could easily pull them back into the normal position. The flexor reflex thresholds of these extremities were usually raised immediately following the operation, but returned to the preoperation level by the 2nd-3rd day.

Experiments on dogs. Five dogs were experimented upon; one dog was used as a control and, of the other four, two had unilateral destruction of the caudate nuclei and two, bilateral while the control dog received only cranial trepanation and surface punctures of the cerebral cortex.

No sharp locomotor disturbances were observed in the dogs Ace and Little Yellow (February 18, 1954 and April 13, 1954) after unilateral damage to the caudate nuclei (0.05 of melted paraffin injected). They walked and ran well. We noted a decrease in the extensor tonicity of the leg muscles on the side opposite the operation which was evident both visually and when the tonicity was measured with Shpigel's apparatus Fig. 2). The flexor reflex thresholds of these extremities to an induced current were raised during the first 2 days after the operation. The deep sensibility of these extremities was disturbed.

The dogs were observed for two weeks after the operation. After this period, the disturbances described had still not yet been fully compensated, but were considerably less expressed. The animals were killed $2\frac{1}{2}$ months after the trauma. Histological examination of the brain showed that a considerable part of their caudate nuclei had been destroyed and that there was cell degeneration in the remaining part (Fig. 3).

In the other two dogs, Gypsy and Mushroom, the caudate nuclei were bilaterally destroyed.

The animals were observed for a long period before the operation, but only one was systematically examined (Mushroom), as the second dog was extremely excitable. Clear conditioned reflexes to light were developed in both dogs, and, in Mushroom, a differentiation was also developed. We used an electric current as an unconditioned reinforcement. Mushroom was operated upon on July 24, 1954 and Gypsy, August 18, 1954.

After the operation, we observed a certain decrease in tonicity and change in the deep and cutaneous sensibility in Mushroom. In Gypsy, stiffness of movement was observed 1-2 weeks after the operation.

The conditioned reflexes were retained in both dogs, although their magnitude decreased somewhat, particularly during the later periods after the operation.

For example, in Mushroom, 2-3 months after the operation, the conditioned reflex was often delayed, sometimes there was no reaction to either the conditioned or unconditioned stimuli; the motions were slow, the paw being raised "in stages" (Fig. 4).

Fig. 3. Frontal section of the left cerebral hemisphere in Ace.

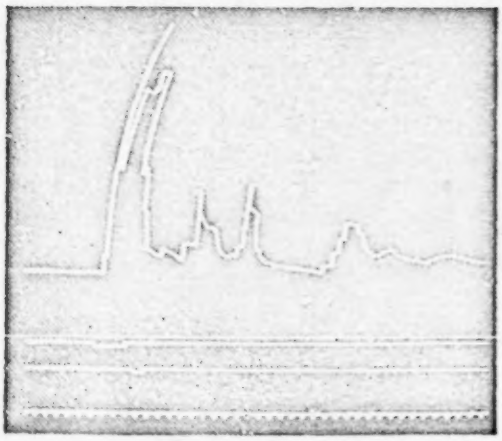

Fig. 4. Graduated character of conditioned motor reaction in Mushroom. Curves from top to bottom show: paw movement, indication of conditioned stimulation, indication of unconditioned stimulation, indication of time (in 1 second marks).

Hyperesthesia to tactile stimulation developed in both animals. No changes were observed in general behavior and locomotion in the control dog (with the cranial trepanation and punctures of the cerebral cortex).

Gypsy was killed 2½ months and Mushroom 7 months after the operation. Pathologicoanatomical and histological examinations of the brain showed that the paraffin had destroyed most of the caudate nucleus in Gypsy's left hemisphere; there was less damage to the caudate nucleus in the right hemisphere, but the remnant of the caudate nucleus was degenerating due to hydrocephalus of the ventricle. In Mushroom, there was pronounced bilateral degeneration of the cellular elements of the caudate nucleus.

We consider the above experimental data obtained from rabbits and dogs to be preliminary. One can nevertheless make certain conclusions from these data with a view to outlining the course of further study. The consequences described which result from the destruction of the caudate nuclei should apparently be considered both as symptoms of disassociation and as symptoms of stimulation – a combination which is often clinically found in various diseases (usually with tumors) of the subcortical ganglia.

SUMMARY

The consequences of destruction of the caudate nucleus were studied in rabbits and in dogs. Injections of molten paraffin into the above subcortical formation were used for that purpose (the puncture was performed through the brain cortex). It was considered that this may bring about disturbances similar to those which appear in brain tumors.

It was demonstrated by the results of these experiments that the delicate function of motor coordination was disturbed in local destruction of the larger portion of the caudate nucleus. Sensitivity, both cutaneous and

proprioceptive was, likewise, disturbed. Characteristic changes appeared in the animals' behavior. Conditioned reflexes were disturbed in rabbits, as well as in dogs.

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VASCULAR PERMEABILITY IN SOME DISEASES OF THE CENTRAL AND PERIPHERAL NERVOUS SYSTEMS

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In our previous investigations [1, 2] it was established that patients with lesions of the diencephalic area and with pain syndromes of various etiology showed considerable changes of permeability in cutaneous capillaries. The criterion for judging permeability in the direction tissue ⟶ blood was the time taken for half-elimination of NaCl solution labelled with Na^{24}, introduced into the skin of the forearm (right and left). The technique used was that developed in I. A. Olvin's laboratory [3]. Allowance was made for the fact that the rate of absorption reflected the functional state of the capillary wall and depended not only on its permeability but also on the extent of absorbing surface and rate of blood flow.

In practically healthy subjects the time of half-elimination of Na^{24} was, on the average, 9.6 minutes both in the right and left forearms. Patients with diencephalic lesions were divided into three groups according to the rate of absorption of Na^{24}: 1) with increased permeability ($3\frac{1}{2}$ - 6 minutes), 2) with lowered permeability (up to 20-28 minutes) and 3) with permeability corresponding to the lower level of the normal range (average of $7\frac{1}{2}$ minutes).

Patients with pain syndromes (radiculitis, neuritis, neuralgia, vascular tumors etc.) exhibited marked asymmetry of the rate of absorption on the healthy and affected sides of the body. Pain syndromes were associated in the majority of cases with lowered permeability; the absorption curve showed a diphasic character slowing markedly during the course of investigation.

The work of L. S. Shtern and collaborators [4] has shown that the composition and properties of tissue fluid depend both on the passage of substances from the blood into the tissues and on the passage of products of metabolism from the tissues into the blood. The relative constancy of extra-cellular fluid can only be maintained when the movement of water, electrolytes and nutrient substances from the blood into the tissues is balanced by a reverse movement from the tissues into the blood. The amount, composition and properties of tissue fluids depend on the capillary wall permeability in both directions.

The present communication, which is one of a series of investigations of the state of cell blood barriers in certain diseases of the nervous system (work of G. N. Kassil and collaborators), is concerned with the results of studies on capillary permeability in the direction blood ⟶ tissues.

EXPERIMENTAL METHODS

$Na^{24}Cl$ with activity of approximately 70 microcurie was given intravenously in 55 ml 20% solution of glucose. Blood samples for determination of activity were taken from the finger (0.1 ml) at intervals of 1, 3, 5, 10, 20, 30, 60, 120, 180 minutes. Intensity of radiation was determined by a counter using installation "B." The rate of passage of Na^{24} from blood into tissues was expressed in percentage of maximal concentration of the isotope in blood, reached directly after its administration (in the course of the first minute).

In the case of control subjects (healthy, young persons) the rate of Na^{24} uptake from the blood by the tissues usually reaches 52% by the 5th minute, 70% by the 20th minute, the latter level being maintained for up to 3 hours.

Observations were carried out on patients with diencephalic lesions (14 cases) and with pain syndromes of various etiologies (15 cases).

Restuls of observations on patients with diencephalic disorders are given in Table 1.

TABLE 1

Rate of Removal of Intradermally Administered $Na^{24}Cl$ in Patients with Diencephalic Syndrome

No. of experiment	Date of experiment, 1956	Subjects	Sex	Age, in years	Removal of Na^{24} from blood (as % of its maximal value) in course of min.								Time of $Na^{24}Cl$ admin. intraderm. absorp. (min)
					3	5	10	20	30	60	120	180	
1	23/XII	S.	F.	20	—	—	5	—	15	20	38	40	5.5
2	22/XII	K.	M.	20	—	—	9	—	40	46	46	46	6.0
3	19/I	F.	»	27	5	—	10	—	25	30	33	40	6.0
4	20/I	M.	F.	32	—	10	—	18	22	33	40	40	11—14.5
5	23 II	K.	»	44	10	—	21	27	—	33	38	—	5.5
6	23/III	P.	M.	30	—	15	—	26	27	46	48	—	5.5
7	9/V	Z.	»	18	5	10	24	30	34	46	—	48	8.0
8	1/III	D.	F.	42	15	—	54	55	60	62	62	—	7.5
9	24/V	M.	»	35	27	39	50	59	60	—	60	—	7.5
10	31/V	G.	»	57	—	20	35	36	55	60	62	—	12.0
11	29/III	B.	M.	53	52	62	66	74	83	83	—	—	20.0
12	6/IV	B.	F.	37	26	50	60	60	88	80	—	—	10.0
13	1/IV	L.	»	32	47	52	53	69	71	72	72	—	20.0
14	31/V	K.	»	33	13	49	56	60	66	—	67	—	10.0

As can be seen from these data, the rate of removal of Na^{24} from the blood was considerably slowed in the case of the first 7 patients. At 20 minutes only 18-30% Na^{24} had been removed, and up to 40-48% subsequently. Slowed removal of Na^{24}, though less marked, was also seen in the next three patients (at 20 minutes 36-59% and then 60-62% was removed). Cases No. 11 and No. 12 showed, in contrast, accelerated removal of Na^{24} which reached, at 5 minutes, 50-62% and 80-83% at 30 minutes. The two last patients showed rates of Na^{24} removal which were within normal limits. Thus, of 14 patients investigated, 10 exhibited slowing of the passage of Na^{24} from blood to tissues and accelerated (except the fourth patient) absorption of Na^{24} from the tissues into the blood (average 5.7 minutes).

The rate of Na^{24} removal from blood is presented graphically in the figure. The curve for Na^{24} removal from blood shows in its first part (up to the 10th minute) the intensity of Na^{24} exchange between blood and extra-cellular fluid, and in its second part the onset of a relative state of equilibrium between these systems. While the normal removal of Na^{24} from blood reaches 70-74% by the 20th minute, in the first group of patients with diencephalic disorders only 18-30% is removed during this time (see Figure) which indicates retention of Na^{24} in the blood. Accelerated absorption of Na^{24} from the tissues into the blood is usually observed in these patients as has already been reported in a previous communication [1]. Clinical and physiological examination of the patients revealed, as a rule, accelerated excretion of water from the organism, dryness of skin and tissues and tendency to wasting. Curve 3 (see Figure) is characteristic for rapid removal of Na^{24} from the blood which is evidently accompanied by its accumulation in extra-cellular fluid. These patients not infrequently show slowed uptake of Na^{24} from the tissues by the blood [1] as well as retention of water in the body, edema and excessive deposition of adipose tissue. The material presented thus indicates that patients

TABLE 2

Rate of Na^{24} Removal in Patients with Pain Syndrome

No. of experiment	Date of experiment, 1956	Subjects	Sex	Age	Removal of Na^{24} from blood (in % of its maximal value) in course of min.								Absorption time in min.	Diagnosis
					3	5	10	20	30	60	120	180		
1	24/XI	K.	M.	57	27	39	50	53	—	55	57	—	15	Right-sided lumbo-sacral radiculitis
2	1/III	P.	»	66	20	—	25	37	42	46	43	43	11	Left-sided lumbo-sacral radiculitis
3	15/III	K.	»	41	—	30	36	39	45	59	61	61	11—20	Trigeminal neuralgia
4	22/III	F.	»	26	23	—	—	—	59	62	—	—	11	Right-sided lumbo-sacral radiculitis
5	10/V	P.	»	70	28	—	35	50	55	58	61	61	19—25	Bilateral lumbo-sacral radiculitis
6	9/II	K.	»	62	30	49	—	70	75	78	78	77	24	
7	24/II	R.	»	31	32	37	49	55	67	70	72	—	13	Vascular pains. Angioneurosis
8	14/III	O.	F.	32	30	56	57	60	66	67	—	67	11	Autonomic polyneuritis. Angioneurosis
9	15/III	L.	M.	32	—	38	59	65	65	70	72	72	9	Chronic headaches — otitic etiology
10	5/IV	B.	»	25	40	52	63	70	70	72	75	—	6	Chronic headaches — angio-neurotic etiology
11	9/V	G.	»	51	—	35	50	60	67	69	69	—	20	Pain syndrome-multiple sclerosis Angioneurosis
12	29/II	M.	F	56	64	—	80	—	82	88	—	88	7	Right-sided lumbo-sacral radiculitis
13	12/IV	T.	M.	33	52	62	65	70	74	83	83	—	6	Thoracic ganglion inflammation. Sympathalgia
14	16/V	C.	F.	46	—	50	62	68	72	77	83	—	25	Pain syndrome — spondyloarthritis
15	24/V	K.	»	45	58	70	75	77	80	85	88	—	9	Severe headaches — angioneurosis

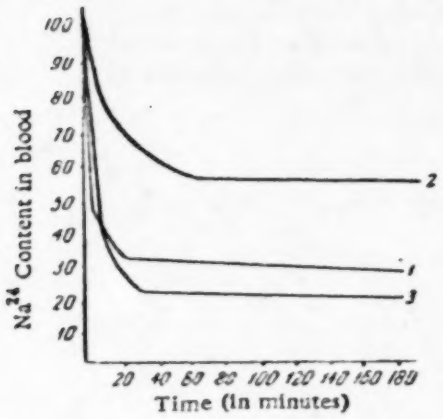
TABLE 3

Absorption Time for Intradermally Introduced $Na^{24}Cl$ in Patients with Diencephalic Syndrome, Following Administration of Sympatol

No. of experiment	Date of experiment	Subjects	Sex	Age	Absorption time in min.	
					before admin. of sympatol	after admin. of sympatol
1	17/IX 1955	P.	F.	26	4.4	16.0
2	12/X	T.	»	33	6.5	12.5
3	23/XI	Kh.	»	42	4.7	7.5—13
4	1/XII	S.	M.	43	5.5	11.0
5	15/XII	S.	F.	22	5.5	5.5
6	15/XII	K.	M.	20	6.0	8.0
7	21/XII	B.	F.	23	6.0	9.5—14
8	29/XII	P.	»	33	3.7	4.5
9	19/I 1956	K.	»	44	5.5	6.0
10	8/III	K.	»	35	6.0	12.5
11	15/III	D.	»	42	9.5	14.0

with diencephalic disorders show definite, consistent changes of vascular permeability both in the direction tissues ⟶ blood and blood ⟶ tissue.

Changes of permeability in both directions were also studied in patients with pain syndromes. The results are presented in Table 2. These data show that patients with lumbo-sacral radiculitis and neuralgias have a diminished rate of Na^{24} removal from blood into tissues. By the 20th minute 37-50% was removed and a state of equilibrium was attained when 49-61% Na^{24} had been removed. Patients with pain caused by various conditions (Nos. 6-11) showed Na^{24} removal rate which was within normal limits (67-78%); in 4 patients (Nos. 12-15) accelerated removal of Na^{24} from blood was observed (83-88%). In 7 patients out of 15 the time of absorption of $Na^{24}Cl$ from the tissues into the blood was considerably prolonged while in the others it was within limits of normal. In this group of patients, unlike those of the diencephalic disorder group, it proved to be impossible to reveal strict dependence of movement of Na^{24} in the direction blood ⟶ tissues and tissues ⟶ blood. It is connected apparently with different etiology of the pain syndrome in various disorders of the central and peripheral nervous systems. The pain syndrome as such does not produce characteristic changes of vascular permeability in the direction blood ⟶ tissues as distinct from the data obtained in studies on permeability in the direction tissues ⟶ blood. It must be supposed that the rate of removal of Na^{24} from the blood depends on the main disease and may be of definite significance for differential diagnosis and therapeutic considerations.



Rate of Na^{24} removal from blood in the normal (1) and in patients with diencephalic disorders (2 and 3). Initial Na^{24} content of blood taken as 100.

Dynamic studies of vascular permeability on some patients revealed that improvement in the general condition under the influence of therapy was accompanied by normalization of the movement of Na^{24} from the blood into tissues and vice versa. In the case of patient K., aged 44 years, with marked manifestations of diencephalic disorder vascular permeability to Na^{24} in the direction of tissues ⟶ blood was increased on first examination, when its removal from the blood was slowed. Following 8 irradiations of the diencephalic area with roentgen rays the vascular permeability to Na^{24} in both directions was normalized against a background of general improvement in the patient's condition.

Taking these data into consideration, it was decided to study the problem of possible directed alteration of capillary permeability. Certain "vegetotropic" substances, widely used in clinical practice, were chosen for study (sympatol, proserine*).

Results of these investigations are presented in part in Table 3.

The data obtained show that absorption time for Na^{24} after preliminary administration of sympatol was considerably increased in most patients, in some cases 2-3 fold. Apparently, the sympathetic reaction which occurs on administration of sympatol and is accompanied by vaso-constriction, rise of blood pressure, acceleration of blood flow and slowing of absorption favors change in capillary permeability.

When proserine was given to the subjects under invesgation it was thought that depression of cholinesterase activity and accumulation of acetylcholine connected with this would cause a parasympathetic effect and, unlike sympatol., lead to acceleration of Na^{24} absorption. However, acceleration of absorption was noted in only 2 out of 7 cases. This was associated with pronounced increase of parasympathetic tonus (slowing of pulse, pupillary constriction, increased peristalsis, nausea). In the remaining cases administration of proserine did not produce any appreciable parasympathetic effect or any changes in the permeability of skin capillaries. Investigations along these lines are being continued.

* Russian trade name.

SUMMARY

Permeability of blood vessels to Na^{24} was studied in the direction of tissues to blood and vice versa. These investigations were conducted in healthy individuals as well as in patients with diencephalic lesions and with pain syndromes of various etiology.

Pronounced delay of removal of Na^{24} from the blood into the tissues was noted in diencephalic patients with increased permeability in tissue to blood direction. A more rapid removal of Na^{24} from the blood was revealed in patients with reduced permeability from the tissues into the blood. The rapidity of Na^{24} removal from the blood into the tissues in the patients with pain syndromes of various etiology is different (its velocity is increased in one case and decreased in the other). Certain vegetotropic substances widely used in the clinic (such as sympatol and proserine) were used for directed change of permeability. It was established that following administration of sympatol absorption of Na^{24} which was injected subcutaneously is greatly delayed.

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BLOOD CLOTTING SYSTEM AND HEMORRHAGE INTO THE BRAIN UNDER CONDITIONS OF NERVOUS TRAUMA

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It is known from the literature that experimental reflex epilepsy of rats, evoked by prolonged auditory stimulation, leads in some cases to the death of the animal, from brain hemorrhage [2,3].

It has also been shown that previous administration of thyroid extract causes a marked rise in mortality of the epileptic rats, with a corresponding rise in the incidence of brain hemorrhage, while previous prolonged treatment with methylthiouracil has the opposite effect of lowering the mortality rate [4].

According to L. V. Krushinsky, L. P. Pushkarskaya, and L. N. Molodkina [2] the basic cause of brain hemorrhages in nervous trauma lies in a profound disturbance of the interaction of stimulatory and inhibitory processes.

It is evident that possible pathological changes in capillary fragility and permeability, blood pressure, blood coagulation, etc., may lead to the inception of hemorrhage.

In this connection, we thought it would be of interest to examine some factors of the blood clotting system, and to ascertain whether changes in their content is related to formation of hemorrhages.

EXPERIMENTAL METHODS

We subjected rats to acoustic stimulation, as in our experiments [2]. The experimental animals were sensitive to acoustic stimuli, i.e., they reacted to the sound of a bell by violent motor excitation, leading in most cases to epileptiform fits. The rats were subjected to intermittent acoustic stimulation for a period of 15 minutes, with intervals of 10 seconds every 10 seconds, and this was followed by a three minute break, after which the bell was rung again for $1\frac{1}{2}$ minutes (if fits appeared before the completion of the schedule, the ringing was stopped earlier).

The excitation developing after the pause was found to be more intense than before the pause. In many cases (about 12% on the average) it terminated in the death of the animal. Death occurred in some cases 20-30 minutes after completing the bell-ringing schedule, and in others after several hours, or even 1-2 days. Brain hemorrhage was found at autopsy in most (90%) of the animals in question.

Blood samples were taken from the jugular vein before the experiment and in different phases of the pathological process. We determined the prothrombin, thrombotropin, and heparin content, by the methods developed in B. A. Kudryashov's laboratory (MGU) [5,6]. The present research is part of a program being conducted in this laboratory.

We have shown [2] that a marked hypothrombinemia is present in rats suffering from experimental reflex epilepsy (with the usual $1\frac{1}{2}$ minute duration of the acoustic stimulus). The prothrombin level fell by 22%, on the average, 15 minutes after exposure to sound, while the thrombotropin and heparin activities did not vary. In view of these findings, it seemed reasonable to suppose that intensification of the nervous trauma

by exposure to prolonged intermittent ringing, causing fatal cerebral hemorrhage in many of the animals, should cause a greater lowering of blood prothrombin, and possibly also of other components of the blood clotting system.

In the first series of experiments we examined the prothrombin and thrombotropin contents of the blood of 38 rats which were subjected to prolonged acoustic stimulation. Blood was sampled before the bell started ringing, after 15 minutes exposure (during the 3 minute pause), after 30 minutes, 45 minutes, 4 hours, and 24 hours. The results obtained are presented in Table 1.

TABLE 1

Changes in Blood Prothrombin and Thrombotropin of Rats Subjected to Prolonged Acoustic Stimulation Means

Component of blood clotting system	Number of experi. animals	% Mortality	Before exposure	After 15 minutes	After 30 minutes	After 45 minutes	After 4 hours	After 24 hours
Prothrombin (%)	38	10.5	94.4	68.9	68.0	70.3	85.2	100.0
Thrombotropin (%)	30	10.0	93.7	90.2	91.4	90.0	93.9	90.7

EXPERIMENTAL RESULTS

As is evident from Table 1, rats displaying an epileptiform reaction to prolonged acoustic stimulation manifested a marked hypoprothrombinemia. The prothrombin level fell by an average of 25.5% within 15 minutes of the commencement of the exposure. The prothrombin level had risen from the low level within 4 hours, and was back to normal within 24 hours.

It is worthy of note that prolongation of the duration of exposure did not lead to any further lowering of prothrombin content below the values found after a 1½ minute exposure [1]. The organism evidently possesses some protective mechanism, which prevents any further lowering of prothrombin level by more than 30-40%, whatever the intensity of the nervous stimulation, since this would lead to a hemorrhagic condition of the animal.

Of the 38 experimental rats 4 died. Death took place within 30 minutes of commencement of exposure to sound. At autopsy, 3 of these rats had macroscopic hemorrhages into the lateral ventricles of the brain. The prothrombin level found in rats shortly before their death was not lowered more than in the survivors (decrease of 36%, 45%, 38%, and 21%). The average fall in prothrombin level is no greater than in those which survived. It may hence be concluded that the lowering of the prothrombin level of the blood of rats subjected to prolonged acoustic stimulation is not a determining factor in the development of the observed hemorrhages, but it may contribute to the action of other factors known to be concerned in the development of cerebral hemorrhage (changes in fragility and permeability of capillaries, in blood pressure, and in other components of the blood clotting system).

A study of blood thrombotropin in 30 rats, under conditions of experimental epilepsy caused by prolonged acoustic stimulation, showed no significant changes. An exception to this finding was provided by two rats which died of cerebral hemorrhage, and which had low thrombotropin levels before death (33% and 39%). We cannot, however, claim that this fall in thrombotropin content contributed in any way to the development of hemorrhage, because of the small number of cases, and because of the possibility of experimental error (blood was taken from moribund animals, and for this reason entered the syringe very slowly). The subject requires further experimental investigation.

The second series of experiments was devoted to a study of blood clotting factors in hyperthyroid rats in conditions of experimental epilepsy, and of rats previously treated with methylthiouracil.

We first examined the effect of thyroid treatment on prothrombin, thrombotropin, and heparin activities of rat blood.

Our experiments showed that in experimental thyrotoxicosis caused by prolonged administration of thyroid preparation (100-150 mg per kg body weight per diem for 10-12 days) the prothrombin level underwent

considerable individual fluctuations. Nevertheless, most of the rats (22 out 39) showed a raised level of prothrombin (in some rats up to 150-160%), on the average on the 7th or 8th day of administration, followed by a fall (in some animals down to 50%). We found no changes in thrombotropin or heparin activities of thyroid-treated rats.

The blood clotting mechanism in experimental thyrotoxicosis of animals has been very little studied. Most of the work on this subject has been done on human subjects with thyroid gland disease. It has been noted, for example, that in various hyperthyroid conditions there is a considerable retardation of clotting, amounting in some cases to a hemorrhagic state, and associated with a fall in prothrombin content [7, 8, 9, 10]. It should, however, be borne in mind that hyperthyroid patients, particularly acute cases, usually display degenerative changes of the liver cells, as a result of which production of the basic components of the blood clotting system is interfered with. It is for this reason that a hemorrhagic diathesis is encountered in severe thyrotoxicosis.

TABLE 2

Changes in Prothrombin Content and Heparin Activity of the Blood of Hyperthyroid Rats, and of Rats after Prolonged Methylthiouracil Treatment, under Conditions of Experimental Epilepsy Caused by Prolonged Acoustic Stimulation
(Mean values)

	Component of the blood clotting system	Number of experimental animals	Percentage mortality	Before thyroid administration	Before acoustic stimulation	30-40 minutes after stimulation
Hyperthyroid rats	Prothrombin, %	16	25.0	91.8	83.4	61.2
	Heparin (seconds)	16	25.0		43.2	44.3
Control	Prothrombin, %	38	10.5		94.4	68.0
Methylthiouracil rats	Prothrombin, %	14	28.6		100.7	73.4
Control	Prothrombin, %	12	58.3		93.2	62.5

A study of the content of basic components of the blood clotting system of hyperthyroid rats under conditions of experimental epilepsy showed that prolonged acoustic stimulation caused pronounced hypoprothrombinemia, observed 30-40 minutes after exposure to the stimulus (Table 2). Blood prothrombin falls by an average of 22%. This fall is, however, no greater than that found in normal "sensitive" rats (controls).

This is evidence that the observed changes in prothrombin level are not determining factors in raising the mortality of hyperthyroid rats, due to cerebral hemorrhage following prolonged acoustic stimulation.

The heparin activity of the blood of hyperthyroid rats is unaffected by prolonged acoustic stimulation.

Four of the 16 experimental rats died after exposure to sound, and 3 of them were found at autopsy to have massive hemorrhages into the lateral ventricles. Shortly before death, these rats had prothrombin levels of 63, 62, 60, and 71%, whereas the surviving rats included some with lower levels (49, 48, 81, 51, 40%).

A pronounced fall in prothrombin content was observed in some rats on the 9-10th day of thyroid treatment (down to 50% in some cases). Prolonged exposure to sound did not cause any change in their prothrombin content, a marked decrease of which was observed in rats whose prothronibin level was close to the normal. This observation also supports the view that animals possess a protective mechanism which prevents the prothrombin level from falling by more than 30-40% on exposure to this type of nervous trauma.

After prolonged methylthiouracil administration* (30 mg per 100 g body weight daily for 10 days) the fall in prothrombin content of the blood after prolonged acoustic stimulation was 30.7%, on the average; this is about the same as for the control group (27.3%). This is evidence that the changes in prothrombin content observed in the methylthiouracil group of rats were not a determining factor in reducing mortality from cerebral hemorrhage, in this group.

Summing up our experimental results, we have established that a pronounced fall in prothrombin level (by an average of 23%) takes place in the blood of rats exhibiting an epileptiform reaction to prolonged acoustic stimulation. The fall in prothrombin content is not, however, so great as to account entirely for the incidence of cerebral hemorrhage in experimental epilepsy.

Certain authors, who have endeavored to elucidate the cause of intracranial hemorrhages of new-born children, have also come to the conclusion that such subarachnoid hemorrhages are not determined by lowering of prothrombin activity [11].

The changes in blood prothrombin of rats due to impairment of nervous regulation are of short duration (the effect of acoustic stimulation appears after a few minutes, and normal levels are again found within a day). It may be supposed that the nervous system exerts a regulatory, compensatory action. This is one of the aspects of the trophic role of the nervous system.

The absence of any change in the thrombotropin content, and the observed changes in prothrombin content of the blood, suggest the existence of different regulatory mechanisms for these two thrombogenic components, which are synthesized basically in the liver. The considerable stability of the thrombotropin level under conditions of nervous trauma can, apparently, be related to the important role played by thrombotropin in the living organism as an initiator of the process of blood coagulation.

SUMMARY

Experimental reflex epilepsy in rats due to prolonged sound stimulation caused death in certain animals due to hemorrhage into the brain. The possible pathological changes in permeability and stability of capillaries, in the blood pressure and in coagulation, etc. may promote the appearance of hemorrhages. It was demonstrated experimentally that in condition of epileptiform reaction in rats caused by prolonged sound stimulation, prothrombin value of the blood shows marked reduction (by 23% on the average). The activity of thrombotropin and heparin does not change materially. Decrease in the level of prothrombin is not large enough to be the fundamental cause of appearance of hemorrhages in rats.

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* The rats used in this series of experiments were supplied from L. V. Krushinsky's laboratory. We determined only the prothrombin content of the blood of these animals.

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ROLE OF THE HYPOPHYSIS IN THE RESISTANCE OF RATS TO DIPHTHERIA TOXIN

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Of the investigations into the role of the hypophysis in the resistance of animals to various pathogenic factors, comparatively few are devoted to resistance to diphtheria toxin [4, 7, 15]. Frick and Lamp [7] found that hypophysectomized guinea pigs have a lower survival rate than do normal animals.

Skuratova (1956) has shown that introduction of ACTH at the time of immunization with diphtheria toxoid raises the resistance of guinea pigs to subsequent injection of diphtheria toxin, as compared with control animals. A similar effect is found when ACTH is injected 3 months before the injection of diphtheria toxin, with subsequent repeated doses of ACTH at various times before injection of toxin, and simultaneously with its injection.

We thought it would be instructive to examine the resistance of albino rats to diphtheria toxin; these animals are reported [8] to be 5000-10,000 times more resistant than guinea pigs.

The object of the present research was to investigate the effects of hypophysectomy and of administration of ACTH to hypophysectomized rats on their resistance to diphtheria toxin.

EXPERIMENTAL METHODS

The animal material consisted of 64 female albino rats, weighing from 140 to 190 g; of these, 48 were hypophysectomized, and 16 were controls. The control animals were subjected to a dummy operation, involving the same operational manipulations, but without removing the hypophysis. Hypophysectomy was performed by a paratracheal approach, described by Silaeva [3]. The daily dose of ACTH (6 mg per 100 g body weight) was dissolved in 1% sodium chloride solution, and injection subcutaneously into the left thigh, in 3 doses, with a 12 hour night interval. The animals were given ACTH during the whole period of intoxication (8-10 days). The first injection of ACTH was given 1 hour before injection of diphtheria toxin. The first series of experiments was performed on 16 controls and 16 hypophysectomized rats. Ten days after the operation both groups of animals were given diphtheria toxin (guinea pig MLD = 0.0026 ml). In the second series of experiments two groups of 16 hypophysectomized mice were taken, 10 days after the operation. The rats of the first group, which served as controls, did not receive ACTH, while those of the second group were given ACTH, according to the above schedule. All rats were then given diphtheria toxin. Only animals in which the total absence of hypophyseal tissue had been confirmed were taken for these experiments. Completeness of hypophysectomy was checked by histological examination of the ablated gland, by visual examination of the hypophyseal cavity, using a binocular lens, by comparison of the absolute eosinophil count of the peripheral blood before and after hypophysectomy, and by the adrenalin test (a modification of Thorn's test [14]). LD_{50} was determined according to Reed and Muench [11].

EXPERIMENTAL RESULTS

The results, presented in the Table, show that LD_{50} for diphtheria toxin is smaller for hypophysectomized than for intact rats (0.247 and 0.418, respectively). It is evident from a comparison of the number of rats dying and surviving that mortality is higher in the hypophysectomized group. The length of the survival time in the two groups also shows that hypophysectomy leads to a fall in resistance to diphtheria toxin.

A similar comparison of the data for hypophysectomized rats receiving and not receiving ACTH indicates that adrenocorticotrophic hormone raises the resistance of hypophysectomized rats to diphtheria toxin.

Hypophysectomy is followed by dysfunction of numerous endocrine glands, and by disorganization of numerous processes concerned with hypophyseal function. In this connection, the regulatory function of the central nervous system is profoundly disturbed [5]. Of the large number of factors leading to lowering of resistance of hypophysectomized rats, we attach special importance to those connected with hypophyseal-adrenal relations, since it is these which, according to numerous authors, are responsible for determining the resistance of the organism to the action of harmful factors.

Resistance of Hypophysectomized and Control Rats to Diphtheria Toxin

Animals \ Indicators of resistance	LD_{50}	Number of rats dying and surviving* — dose of toxin, in ml per 100 g body weight						Mean survival time (days)
		0.615	0.473	0.364	0.28	0.21	0.16	
Intact	0.418	4/0	3/1	1/3	0/4	0/4	—	7.8
Hypophysectomized	0.247	4.0	4.0	7/1	4/4	1/3	1/3	6.1
Hypophysectomized, given ACTH	0.300	--	—	4/0	3/1	2/2	0/4	7.4

* Numerator: number of rats dying; denominator: number surviving.

The protective effect of the adrenals in diphtheritic intoxication was shown by Bogomolets [1], Molchanov [2], and, later, by Lewis and Page [9], Yurkov [6], and others.

It is noteworthy that corticoadrenal extracts and individual corticosteroids are effective in raising the resistance of adrenalectomized rats only, to diphtheria toxin, but have no significant effect in intact animals [8, 9].

Of the individual corticosteroids, only glucocorticoids (compound A acetate) raise the resistance of adrenalectomized rats to diphtheria toxin; desoxycorticosterone acetate was without effect [9].

According to a number of authors [12, 14], the adrenocorticotrophic hormone of the anterior hypophysis is active chiefly in stimulating secretion of glucocorticoids.

If we accept Selye's view [13] that the hormone reaction is triggered by ACTH secretion, it would follow that the protective action of the adrenal cortex could not be fully developed in the absence of the hypophysis. This supposition is supported by the finding that administration of ACTH to hypophysectomized rats raises their resistance to diphtheria toxin. Skuratova's findings [4] also supports this view.

Our experimental findings support the view that the hypophysis plays a significant part in the determination of the natural resistance of rats to diphtheria toxin. It is probable that this protective effect is mediated through its adrenocorticotrophic function. Other pituitary hormones may of course, also exert a protective action, since ACTH administration raises the survival rate of hypophysectomized rats by only 10%, as compared with hypophysectomized rats not receiving ACTH, whereas hypophysectomy lowers their resistance by over 40%.

SUMMARY

It was established that hypophysectomy causes decreased resistance of rats to diphtheria toxin. LD_{50} of diphtheria toxin is less for hypophysectomized rats than for intact ones. It equals correspondingly 0.247 and 0.418. Introduction of ACTH to hypophysectomized animals increased their resistence to diphtheria toxin (LD_{50} equaled 0.303).

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INCORPORATION OF RADIOACTIVE S^{35}-METHIONINE AND C^{14}-GLYCINE INTO THE PROTEINS OF WHOLE LIVER TISSUE AND OF LIVER CELL NUCLEI, AT VARIOUS TIMES AFTER PARTIAL HEPATECTOMY

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Researches conducted in the Biochemical Laboratory of the P. A. Gertsen Oncological Institute [2, 4, 9] have shown that tumor cell nuclei differ from those of ordinary tissues in the relative proportions of their protein fractions. Malignant tumor cell nuclei show a pronounced rise (by a factor of 5-10) in the content of residual protein fractions, at the expense of nucleoprotein and acid protein fractions. In order to ascertain the specificity of these changes, a study was made of the composition of the cell nuclei of other rapidly growing tissues (embryonic and regenerating) [3]. During the early stages of compensatory growth of liver cells following partial hepatectomy the cell nuclei of regenerating tissue to a certain extent resemble, as regards their protein fractions, the nuclei of tumor cells. This allows us to draw a parallel between the dedifferentiation of tissues, taking place at this stage of regeneration, and the anaplasia of tumors [3].

Experiments on the incorporation of labelled amino acids into whole tissue proteins and into the nuclei of malignant tumor cells in vivo showed that the nuclear proteins of tumor cells took S^{35}-methionine and C^{14}-glycine 3-4 times more slowly than did the whole tissue proteins or the nuclear proteins of normal liver [5]. It remains to be shown to what extent this phenomenon is characteristic of tumor tissue, or whether it applies to all rapidly growing tissues, and whether it bears any relation to differentiation of tissues.

The present paper is devoted to a study of the rate of incorporation of S^{35}-methionine and C^{14}-glycine into the proteins of whole tissue and cell nuclei at various stages of compensatory growth following partial hepatectomy.

EXPERIMENTAL METHODS

The abdominal cavity of rats weighing 130-150 g was opened under ether anesthesia, and a part of the liver was ligated and excised. At various times after the operation (2, 5, 10, 15, and 20 days) injections of S^{35}-methionine were given subcutaneously, at a dosage of 3000 to 6000 impulses per minute per g body weight. The animals were killed by decapitation 30 minutes and 3 hours after the injection, and the liver was taken for examination, after trimming off the necrotic parts. The cell nuclei were isolated by Dounce's method, at pH 6.8 [12]. In some of the experiments the nuclei were isolated in a 1% citric acid medium [13]. Parallel with this we performed a series of experiments on the incorporation of labelled amino acids into the proteins of liver slices in vitro. The experiments were conducted according to the method described in an earlier paper [6].

The proteins were processed in the usual way. Radioactivity was measured by means of a Geiger counter, on a 10 mg sample of protein spread evenly over the surface of aluminium discs, diameter 18 mm. The results were calculated as the ratio of impulses per minute per g of protein and per g body weight.

TABLE 1

Incorporation of S^{35}-Methionine into the Proteins of Whole Tissue, Nuclei, and Nuclear Fractions of Regenerating Rat Liver at Various Times after Partial Hepatectomy. Three Hours After Injection of Radiomethionine. The Nuclei were Isolated in Neutral Solution. Impulses per min. per g of Protein; Impulses per min. per g Body Weight

Experiment	№ 9				№ 11			№ 14						№ 16					
	days after partial hepatectomy																		
	0	10	15	20	2	4	11	2	4	11	14	2	6	10	15	19
Whole tissue proteins	3.4	3.4	5.6	6.2	8.0	6.2	9.3	5.1	5.1	3.3	3.9	9.5	7.2	4.8	5.4	5.3
Nuclear proteins	1.0	3.3	7.1	7.2	5.7	3.6	8.1	4.9	3.5	—	3.8	8.0	5.5	6.5	4.7	5.3

EXPERIMENTAL RESULTS

Our experiments on the incorporation of S^{35}-methionine into the proteins of whole liver tissue and into the cell nuclei of regenerating liver of rats showed (Table 1) that the highest radioactivities were found in the proteins of whole liver tissue during the first few days after partial hepatectomy (for example, on the second day), after which the activity fell, and again rose in the terminal stages of regeneration. On the 2nd day after partial hepatectomy the nuclear proteins incorporate S^{35}- methionine at a somewhat slower rate than do those of whole tissue. By the 4 – 6th day the radioactivity of the nuclear proteins had fallen by $1\frac{1}{2}$ – 2 times, and it regained its earlier level only on the 15 – 20th day after the operation.

Fractionation of the nuclear proteins [4] was performed in some of the experiments only, owing to the insufficient amounts of available material.

The results of these experiments showed that the highest radioactivity was found in the acid protein fraction, and the lowest in the residual proteins.

The data for incorporation of C^{14}-glycine into the proteins of regenerating liver tissue are presented in Table 2. In these experiments the cell nuclei were isolated in acid medium; inclusion of radioactivity into the nuclear proteins at different stages of regeneration did not differ appreciably from that for whole tissue proteins.

Our results for rate of incorporation into tissue slices in vitro confirmed the findings reported in the literature, that regenerating liver tissue takes up labelled amino acids faster than does normal liver tissue (Table 3).

Since we found, in our earlier experiments [6], that products of hydrolysis of ribonucleic acid had an activating effect on incorporation of labelled amino acids into the proteins of normal liver slices, and that this effect did not apply to tumor tissue, we thought it would be of interest to repeat this experiment with slices of regenerating liver at various times after partial hepatectomy.

Slices of regenerating liver, weighing 150-200 mg, were immersed in a medium containing C^{14}-glycine and 0.05% of ribonucleic acid hydrolysis products.

The results of this experiment (Table 3) showed that the rate of incorporation of C^{14}-glycine into proteins of tissue slices taken on the second day after partial hepatectomy was unaffected by ribonucleic acid hydrolysis products.

TABLE 2

Incorporation of C^{14}-glycine into the Proteins of Whole Tissue and Cell Nuclei of Rat Livers, Isolated in Acid Medium, at Various Times after Partial Hepatectomy, 3 Hours after Introduction of C^{14}-Glycine expressed as impulses per min. per g of protein : impulses per min. per g weight

Experiment	№ 20				№ 21				
Day after hepatectomy	2nd	6-th	9-th	20	3-rd	6-th	10-th	14-th	20-th
Whole tissue	5.5	3.7	3.6	2.7	4.7	4.1	3.7	2.9	2.9
Nuclei	6.2	3.7	3.6	3.9	4.6	4.2	2.7	2.7	2.7

TABLE 3

Incorporation of C^{14}-Glycine into the Proteins of Regenerating Rat Liver Slices, at Various Times after Partial Hepatectomy, and the Effect on this Process of Hydrolysis Products of Ribonucleic Acid

Days after hepatectomy / Incubation conditions*	2-d	6-d	14-d
Without addition of ribonucleic acid hydrolyzate	1.17	1.07 1.07	1.1 1.1
In presence of ribonucleic acid hydrolyzate	0.89 1.14	1.33 1.67	2.7 2.9 2.5

* The slices were incubated in Robinson's medium [14] for 50 minutes at 37.8°, in an atmosphere of O_2.

A slight activating effect became apparent on the 6th day, and on the 14th day addition of RNA hydrolyzate raised the rate of incorporation of labelled amino acid by $2 - 2\frac{1}{2}$ times.

It appears from our findings that the synthesizing activity of the nucleus varies at different stages of the regeneration process. Thus the rate of incorporation of labelled amino acids into nuclear proteins is lower on the 4 – 6th day after partial hepatectomy than into whole tissue proteins. It should be noted that at this stage of regeneration, characterized by undifferentiated growth, the cell nuclei to some extent resemble those of tumor cells, the proteins of which incorporate labelled amino acids 3 – 4 times more slowly than do those of whole tissue [5]. At a late stage of regeneration (15 – 20th day), the relative rate of incorporation of amino acids into cell nuclei rises. At this stage, nuclear proteins incorporate S^{35}-methionine at a rate equal to that for whole tissue proteins, and in this respect they may be said to resemble the proteins of normal liver cell nuclei.

Somewhat different results were found for incorporation of C^{14}-glycine into liver regenerate proteins, and in particular into the proteins of the cell nuclei, when these were isolated in an acid medium. In this case we

could find no difference between the rates of incorporation into whole tissue and nuclear proteins.

As was shown by our previous researches [7], when nuclei are isolated in acid media a certain amount of basic proteins, the radioactivity of which is low, is extracted into the solution, as a result of which the radioactivity of the remaining proteins rises, and approaches that of whole tissue proteins.

Our in vitro experiments confirm published reports that the rate of incorporation of labelled amino acids into the proteins of regenerating liver slices is higher than for normal liver, but lower than for tumor tissues.

According to our data, the radioactivity of the proteins of regenerating liver may be expressed in relative units as 1.1, of normal liver as 0.66, of M-1 rat sarcoma as 2.5, and of Ehrlich mouse ascites tumor as 7.8 [6].

During the first few days after partial hepatectomy addition of ribonucleic acid hydrolyzate to regenerating liver slices has no effect on the rate of incorporation of amino acids into their proteins. Much the same was found to apply to M-1 rat sarcoma slices [6]. At later stages of regeneration, addition of ribonucleic acid hydrolyzate raises the rate of incorporation of amino acids by a factor of $2-2\frac{1}{2}$. This finding is in accordance with our earlier data for normal liver slices [6].

Thus the results of our research are consistent with published findings [1, 3, 8, 10, 11], and point to biochemical peculiarities of two stages of regeneration. Although the differences between the rates of incorporation of labelled amino acids into nuclear and whole tissue proteins of liver regenerates of the first stage of regeneration are not great (a factor of $1\frac{1}{2}-2$, as compared with $3-4$ for tumor tissue), we may venture to suggest that there is some resemblance, in this respect, between the early stages of regeneration, distinguished by undifferentiated growth, and the anaplasia of tumor tissue.

Our data also indicate that at a late stage, that of differentiation, the proteins of regenerating liver resemble those of normal liver, as regards the rate of incorporation of S^{35}-methionine.

We regard the results of the present research as affording confirmation of the assumption that the nucleus is responsible for the synthesis of the more specific proteins, which determine the normal development and differentiation of the liver [5]. It may be concluded from our experiments that on the $4-6$th day after partial hepatectomy the liver cell nuclei incorporate S^{35}-methionine at a rate $1.3-2$ times slower than do the whole tissue proteins of the regenerate. On the $15-20$th day after partial hepatectomy the rate of incorporation of S^{35}-methionine is the same for nuclear and whole tissue proteins. Ribonucleic acid hydrolyzates do not affect the rate of incorporation of C^{14}-glycine into the proteins of liver regenerate slices taken on the 2nd day after partial hepatectomy, but they raise the rate of inclusion into tissues taken on the 6th day, to a small extent. By the 14th day the hydrolyzates raise the rate of inclusion of C^{14}-glycine by a factor of $2-2\frac{1}{2}$.

SUMMARY

The rate of inclusion of S^{35}-methionine and C^{14}-glycine into the proteins of whole tissue and cellular nuclei of rats liver at various periods following partial hepatectomy was investigated. It was established that on the 4th to 6th day following partial hepatectomy the process of inclusion of S^{35} into the cellular nuclei of the liver was 1.3 to 2 times slower than in the proteins of the tissue regenerate as a whole. The rate of inclusion of S^{35}-methionine into the proteins of the nuclei and the whole tissue of regenerating liver becomes equal on the 15th-20th day. On the second day after partial hepatectomy the products of hydrolysis of ribonucleic acid have no effect on the rate of inclusion of C^{14}-glycine into the proteins of sections of liver regenerate in vitro. A certain increase of inclusion is noted on the 6th day. Hydrolyzate of ribonucleic acid enhances inclusion of C^{14} from 2 to $2\frac{1}{2}$ times on the 14th day after the operation.

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EFFECT OF ULTRAVIOLET IRRADIATION ON THE PERMEABILITY TO PROTEINS, RADIOCALCIUM, AND RADIOPHOSPHORUS OF THE CAPILLARIES OF THE ANTERIOR PORTION OF THE EYE

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Apart from its theoretical interest, the study of the effect of ultraviolet radiation on the permeability of the capillary vessels of the eye may be of some importance clinically, since it is the cause of the condition known as electro-ophthalmia. The pathogenesis of this condition has not been sufficiently clarified. In particular, we were unable to find any references in the literature to capillary permeability in this disease.

Patients suffering from this condition complain of unbearable pain in the eyes, with photophobia and lacrimation. The symptoms appear after exposure of the unprotected eye to certain sources of light, such as the quartz-mercury vapor lamp in autogenous or arc welding, and in foundry practice during pouring of molten metals, etc. Electro-ophthalmia is analogous to snow-blindness, caused by the sun's rays in mountainous or polar regions. It is also encountered after exposure to the ultraviolet radiation released during nuclear explosions.

The present paper presents the results of a study of capillary permeability after ultraviolet irradiation of the eyes.

Capillary permeability was assessed from data for penetration of certain substances, viz., proteins, P^{32}, and Ca^{45}.

Capillary permeability can be assessed from the protein content of the aqueous humor, which is normally 200-300 times smaller than in the plasma of healthy animals. Augmentation of capillary permeability leads to a marked rise in the protein content of the aqueous humor. We estimated the protein content by a nephelometric method [11], especially adapted to this purpose.

Aqueous humor samples were taken by means of a fine hypodermic needle inserted into the anterior chamber.

Solutions of $Na_2HP^{32}O_4$ and $Ca^{45}Cl_2$ were injected into the marginal vein of the left ear of rabbits. The solutions were injected one immediately after the other: radiocalcium at a dosage level of 1500 imp./g, and phosphorus 200 imp./g. Puncture of the anterior chamber was effected one hour after the injections, and a blood sample was taken simultaneously from the marginal vein of the right ear. The content of both radioisotopes was determined in the same sample of aqueous humor (0.1 ml) or blood (0.01 ml), using filters [9]. The measurements were made in duplicate on dried films of the fluids. We first measured the total isotope activity, using an end-window counter. The measurement was then repeated, interposing a filter which cut off all the Ca^{45} radiation, and half of the P^{32} radiation. This filter consisted of 37 layers of aluminum foil. Counting of impulses was done in a B equipment, from an end-window counter in a lead chamber.

In view of the importance of vitamin C deficiency in the development of disturbances of vascular permeability, we also determined ascorbic acid in the aqueous humor.

Experimental electro-ophthalmia was produced by irradiating the left eye for 30 minutes, using mercury vapor lamps PRK-2 or PRK-4. The distance from the light source to the eye was 65 cm for PRK-2, and 40 cm for PRK-4. The right eye was shielded from the light of the lamps.

EXPERIMENTAL RESULTS

Conjunctivitis and blepharospasm were observed on the next day, and also 2-3 days after irradiation. The sequelae of irradiation were no longer visible after 1-3 weeks.

The parameters measured varied parallel with the clinical changes. The protein content of the aqueous humor of the irradiated eye rose abruptly after 1-3 days (Table 1); Whereas the protein content of aqueous humor of control animals varied from 39 to 84 mg-% [7], after irradiation the range was 200-1840 mg-%.

TABLE 1

Protein and Ascorbic Acid Contents of the Aqueous Humor (mg %) after Irradiating the Left Eye, from a Quartz-Mercury Vapor Lamp

No. of rabbit	Interval bet. irrad. and puncture (days)	Protein		Ascorbic acid	
		right eye	left eye	right eye	left eye
1	1	26	210	17.2	10.2
2	1	40	200	17.3	15.0
3	2	52	450	13.8	6.6
4	2	65	390	24.9	17.1
5	2	49	250	25.7	21.3
6	2	22	240	15.6	13.0
7	3	62	1840	14.6	6.0
8	3	88	520	20.2	11.0
9	3	24	220	21.1	16.3
Average		48	480	18.9	12.9
5	18	59	48	27.4	27.1
4	20	49	35	23.2	22.8
10	23	38	43	18.4	18.7
3	41	45	47	11.8	9.3
7	44	76	72	25.3	26.0
1	45	52	55	18.0	16.9
Average		53	50	20.7	20.1

The protein content of the aqueous humor of the shielded eye did not rise (22-88 mg %).

After a lapse of 18-45 days from the beginning of the process the protein content of the irradiated eye was the same as for normal rabbits.

At the stage of maximum rise in protein content (1-3 days) the aqueous humor of the affected eye showed a higher concentration of radioisotopes than did the unaffected one (Table 2). The mean Ca^{45} content on the irradiated side was 136.8 imp./min., as compared with only 101.4 imp./min. on the opposite side. The corresponding values for P^{32} were 75.2 and 51.2 imp./min., respectively. The mean P^{32} activity of the blood of these rabbits was 148.6 imp./min (range 70-306.9 imp./min). The mean Ca^{45} of the blood was 131 imp./min (range 79-216 imp./min).

Our experimental findings are evidence of increased capillary permeability, for all the substances examined.

The ascorbic acid level fell in many cases to less than half the value found for the aqueous humor of the

TABLE 2

P^{32} and Ca^{45} Activities of the Aqueous Humor (imp./min per 0.1 ml) after Irradiation of the Left Eye with a Quartz-Mercury Vapor Lamp

No. of rabbit	Interval bet. irrad. and puncture	Radiocalcium		Radiophosphorus	
		right eye	left eye	right eye	left eye
11	1 day	126.7	166.1	55.6	105.8
12	1 »	124.0	190.5	73.5	103.6
13	1 »	88.9	124.2	50.4	77.0
14	2 days	51.7	90.1	47.9	60.1
15	2 »	74.4	90.1	31.0	44.3
16	3 »	142.5	159.9	48.7	60.8
Average		101.4	136.8	51.2	75.2

intact eye, at the height of the rise in capillary permeability (Table 1). Such marked asymmetry is not encountered in healthy control rabbits [7]. Nor was it observed in these animals a few weeks after irradiation.

Since the quartz-mercury vapor lamp emits not only ultraviolet, but also visible light (49.5% of total emission [1]), we examined these fractions separately to ascertain whether visible or ultraviolet light was responsible for the changes observed. For this purpose we interposed a glass filter, which cut off all radiation of wavelength less than 334 mμ, between the light source and the eye.

TABLE 3

Protein and Ascorbic Acid Contents (mg %) of Aqueous Humor after Irradiation of the Left Eye with a Quartz-Mercury Vapor Lamp Through a Filter Cutting Off Ultraviolet Light

No. of rabbit	Interval between irradiation and puncture (days)	Protein		Ascorbic acid	
		right eye	left eye	right eye	left eye
17	1	44	31	17.4	19.0
18	1	36	39	19.8	20.2
19	2	34	43	38.9	40.0
20	2	34	37	16.4	17.0
21	3	42	32	14.6	17.0
22	3	44	27	20.2	18.2
Mean		39	35	21.2	21.9

We did not observe cases of electro-ophthalmia after exposure to the screened light, and we found no appreciable changes in the permeability of the capillaries of the eye to the substances studied. The results obtained from these experiments are presented in Tables 3 and 4.

These results are evidence that the changes in permeability of the capillaries following quartz-mercury vapor lamp irradiation of the eye were due to ultraviolet rays of wavelength less than 334 mμ.

The observed rise in protein content of the aqueous humor is due to raised capillary permeability. It cannot be ascribed to passage of proteins from the injured cornea to the aqueous humor, since electrophoresis

experiments have shown [10, 15] that the aqueous humor contains albumins and globulins, of which there is very little in the cornea [13].

We believe that the variations in vitamin C level observed after ultraviolet irradiation are also to a certain extent connected with disturbances of capillary permeability. The aqueous humor normally has an ascorbic acid content 10-30 times higher than that of blood plasma. If capillary permeability rises, vitamin C will diffuse out from the aqueous humor into the blood, as a result of which its concentration falls in the aqueous humor. Lowering of the vitamin C content of the anterior chamber leads, in turn, to still greater disturbance of capillary permeability.

TABLE 4

P^{32} and Ca^{45} Activities of the Aqueous Humor (imp./min per 0.01 ml) after Irradiation of the Left Eye with a Quartz-Mercury Vapor Lamp Through a Filter Cutting Off Ultraviolet Light

No. of rabbit	Interval between irradiation and puncture (days)	Radiocalcium		Radiophosphorus	
		right eye	left eye	right eye	left eye
23	1 day	77.2	69.5	67.5	61.8
24	1 »	95.0	87.2	46.4	45.8
25	1 »	111.4	107.1	31.5	33.2
26	2 days	105.5	97.0	51.2	47.2
27	2 »	71.3	63.2	58.3	61.4
28	3 »	74.0	78.0	39.4	45.4
Mean		89.1	83.7	49.0	49.1

Ultraviolet irradiation of tissues leads to release of histamine and histamine-like substances [6]. The increased permeability of the capillaries of the anterior chamber observed by us may depend on the formation of such biologically active products. This view is supported by the observation that repeated subconjunctival injections of histamine lead to a raised protein content of the aqueous humor, with a simultaneous fall in its vitamin C content [4].

It may also be supposed that the changes observed by us are connected with stimulation of receptors (sensory terminations of the trigeminal nerve, for example). As is known, stimulation of this nerve causes a pronounced rise in the permeability of the capillaries of the eye [5], in particular to P^{32}, Ca^{45}, and protein [3, 8]. Support is afforded to the reflex mechanism by the finding that the nature of the erythematous reaction to ultraviolet irradiation is greatly modified by narcosis [2], and that it proceeds differently in individuals suffering from various lesions of the central nervous system than it does in normal subjects [1].

SUMMARY

Permeability of eye capillaries following irradiation by quartz-mercury lamp was studied by determining the permeation of protein P^{32} and Ca^{45} into the aqueous humor of the eye from the blood of rabbits. The content of proteins, P^{32} and Ca^{45} increased in the aqueous humor of the eye during marked manifestation of electroophthalmia. At the same time, the level of vitamin C was reduced. This showed that the permeability of eye capillaries was increased. One group of rabbits was irradiated by a quartz-mercury lamp protected by a filter which did not allow ultraviolet rays of the wavelength shorter than 334 mμ to pass. However, visible rays were not stopped by this filter. This was done since besides ultraviolet rays the quartz-mercury lamp irradiates numerous visible rays. Conditions of the latter experiment did not cause ophthalmia and had no effect on the above indices. Consequently, it was concluded that increased permeability of capillaries was caused by ultraviolet rays. A theory has been suggested to account for the reflex character of these changes and the significance of histamine in their development.

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SOME ACETYLATION PROCESSES IN THE NORMAL HUMAN ORGANISM AND IN FUNCTIONAL DISTURBANCES OF THE NERVOUS SYSTEM

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Pantothenic acid deficiency in the human [2, 3] has been associated with symptoms of adrenal insufficiency, of impaired activity of the alimentary tract, and of lesions of the nervous system.

The biochemical basis of these pathological changes is related to the functions of coenzyme A (CoA), which is concerned in the synthesis of citric acid [9], in acetylation of amines, including choline, to give acetylcholine [6, 8], in fatty acid metabolism [7], in steroid synthesis [4], and in formation of peptide bonds [1].

The appearance of functional disturbances of the nervous system of human subjects suffering from pantothenic acid deficiency led us to investigate certain biochemical transformations connected with CoA, in patients suffering from neuroses.

We examined: 1) the acetic acid content of the blood, as a possible source of active acetate, 2) percentage acetylation of sulfanilamide, as an index of CoA activity, and 3) the blood citric acid content, since formation of citric acid is closely bound up with CoA activity.

We examined patients suffering from functional disturbances of the nervous system (hysterical neurosis, neurasthenia, and other neuroses). For normal values we took those found for a group of normal persons. This group corresponded in age, sex, and period of observation with the neurotic group.

Blood acetate was determined for groups of 30 normal and 30 neurotic individuals, blood citric acid in 42 normals and 59 neurotics, and percentage acetylation of sulfanilamide in 44 normals and 68 neurotics.

EXPERIMENTAL RESULTS

The results, subjected to statistical treatment, are presented in Tables 1, 2, and 3*, for healthy normals, for patients suffering from hysteria and from neurasthenia, and for the whole group of neurotic patients, including, apart from hysteria and neurasthenia, a small group of other neuroses.

The range of values found was: healthy controls 0-17 mg %, patients 0-18 mg %.

It is evident from Table 1 that the differences found between blood acetic acid content of healthy and neurotic individuals are not statistically significant, although the mean values found for hysterical patients are higher than for the other groups.

The mean citric acid content of the blood of neurotics was much higher than in the control group. This difference is statistically significant (Table 2).

The individual variations found were: controls 0.8-1.6 mg %, neurotics 0.4-1.5 mg %.

* The symbols at the heads of the columns of Tables 1, 2, and 3 represent: n) number of determinations (persons), M) arithmetic means of the values, σ) standard deviations, m) mean error, t) coefficient of significance of differences, P) statistical probability of significance of difference from the values for healthy controls, as per cent.

TABLE 1

Acetic Acid Content (mg %) of the Blood of Normal and Neurotic Individuals

Group examined	*n*	*M*	σ	*m*	*t*	*P*
Healthy	30	6.77	±3.8026	±0.69	—	—
Hystericals	15	8.47	±4.8683	±1.26	1.2	87
Neurasthenics	8	6.88	±5.3301	±1.89	0.1	54
All forms of neurosis	30	7.63	±5.2621	±0.96	0.7	76

The individual variations for percentage acetylation of sulfanilamide found were: healthy controls 10-39, patients 4-27.

The mean values for percentage acetylation of sulfanilamide were lower for the neurotic group than for the controls. The difference is statistically significant (Table 3).

TABLE 2

Blood Citric Acid Content (mg %) of Normal and Neurotic Individuals

Group examined	*n*	*M*	σ	*m*	*t*	*P*
Healthy	43	1.18	±0.1874	±0.03	—	—
Hystericals	27	0.90	±0.1952	±0.04	5.6	100
Neurasthenics	24	0.90	±0.2350	±0.05	4.8	100
All forms of neurosis	59	0.91	±0.2278	±0.03	6.4	100

TABLE 3

Percentage Acetylation of Sulfanilamide in Normal and Neurotic Individuals

Group examined	*n*	*M*	σ	*m*	*t*	*P*
Healthy	44	20.82	±6.1806	±0.93	—	—
Hystericals	30	14.57	±5.6000	±1.02	4.5	100
Neurasthenics	27	16.00	±6.7313	±1.30	4.5	100
All forms of neurosis	68	15.63	±5.8438	±0.71	4.4	100

The simultaneous lowering of the citric acid content of the blood and of percentage acetylation of sulfanilamide encountered in functional disorders of the nervous sytem may be evidence of deficiency of dietary pantothenic acid, or of disturbances in its synthesis in or assimilation from the alimentary tract (percentage acetylation of sulfanilamide was always lowered in neurotic patients when they also suffered from disease of the alimentary tract), or of impairment of synthesis of acetyl CoA.

The experimental evidence does not permit to state which disturbance of pantothenic acid metabolism is present in patients suffering from neuroses, but there can be no doubt that this metabolism is disturbed.

SUMMARY

The content of acetic and citric acid and percentage of acetylation of sulfanilamide were determined both in healthy individuals and patients with neurosis. Metabolism of these substances takes place with the aid of coenzyme of acetylation (CoA), which contains pantothenic acid. The average content of acetic acid in the blood of neurotic patients is nearly normal except in cases of hysteric patients in whom it is slightly increased. Simultaneous decrease of content of citric acid in the blood and the percentage of acetylation of sulfanilamide, which is noted in the functional disturbances of the nervous system may point to deficiency of pantothenic acid in the diet , to disturbance of its synthesis or its assimilation in the digestive tract and to disturbance of the synthesis of acetyl CoA. Evidence at our disposal still does not permit to decide the nature of the metabolic disturbances which take place during neuroses.

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EFFECT OF ANTIBACTERIAL CHEMOTHERAPEUTIC PREPARATIONS OF THE SYNTHESIS OF p-AMINOHIPPURIC ACID IN RAT LIVER SLICES

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The most interesting aspect of the action of bactericidal chemotherapeutic preparations is that of their effect on reactions of synthesis, particularly of proteins.

For this reason we thought it would be of interest to study the effect of chemotherapeutic preparation on the formation of peptide bonds.

For this purpose we applied the model reaction of synthesis of p-aminohippuric acid (PAHA) by rat liver slices from p-aminobenzoic acid (PABA) and glycine [2].

The experimental methods used were described in our previous paper [1]. We compared the amount of PAHA synthesized by liver slices from the same animal, after incubation for 5 hours, with and without addition of the chemotherapeutic substance.

We examined the effects of the antibiotics streptomycin, penicillin, chloromycetin, and biomycin, and of the antituberculosis drugs Tubazid, Ftivazid, and Tibon.*

In those cases in which the amount of antibacterial drug added exceeded its solubility in the medium we added it in the form of a fine suspension.

EXPERIMENTAL RESULTS

The results given by our experiments are presented in Tables 1, 2, and 3.

It appears from the data of Table 1 that Tubazid, Ftivazid, and Tibon do not significantly affect the synthesis of PAHA by liver slices.

The data of Table 2 show that penicillin in high concentrations (100 – 10,000 units per ml) inhibits synthesis of PAHA. At concentrations such as are achieved in the organism for therapeutic purposes we could find no interference with biosynthesis. Streptomycin sulfate depresses synthesis at a concentration of 4000 units per ml, while streptomycin–calcium chloride complex either has no action, or stimulates synthesis, even at very high concentrations.

Table 3 shows that chloramphenicol, syntomycin, dextromycetin, and chlortetracyclin suppress synthesis of PAHA by rat liver slices, at concentrations of 1000 μg/ml. Total inhibition of biosynthesis of PAHA is achieved by concentrations of 10,000 μg/ml of chloromycetin and syntomycin.

DISCUSSION OF RESULTS

Our experiments show that the antituberculosis chemotherapeutic drugs Tibon, Ftivazid, and Tubazid, as well as streptomycin, do not inhibit the reaction of biosynthesis of PAHA, even when taken in very high

* Transliteration of Russian – Publisher's note.

TABLE 1

Effect of Chemotherapeutic Antibacterial Drugs on Synthesis of PAHA by Rat Liver Slices

Serial No.	Name of drug	Weight of liver tissue (g)	Synthesis of PAHA, as % of control, in presence of the drug (μg/ml)					
			10 000	5 000	1 000	100	10	1
1	Tubazid	0.2	—	—	97	—	82	—
2	Ftivazid	0.2	—	—	90	—	100	—
3	Tibon	0.2	84	88	—	86	—	—

TABLE 2

Effect of Penicillin and Streptomycin on Synthesis of PAHA by Rat Liver Slices

Serial No.	Antibiotic	Weight of liver tissue (g)	Synthesis PAHA as % control in presence of antibiotic (units per ml)											
			10 000	5 000	4 000	1 000	500	400	100	50	40	5	1	0.1
1	Penicillin (sodium salt)	0.2	0	—	—	34	—	—	76	—	—	—	—	113	95
2	Crystalline penicillin (K salt)	0.2	2	—	—	—	—	—	76	—	—	—	—	—	—
3	Ditto	0.2	15	—	—	50	—	—	79	—	—	—	—	94	94
4	Streptomycin sulfate	0.2	—	—	64	—	—	—	—	—	93	—	89	—	—
5	Streptomycin-calcium chloride complex	0.2	—	109	—	—	119	119	—	145	—	102	—	—	—
6	Ditto	0.2	—	109	—	—	137	—	—	123	—	109	—	—	—

TABLE 3

Effect of Antibiotics on Synthesis of PAHA by Rat Liver Slices

Serial No.	Name of antibiotic	Weight of liver tissue (g)	Synthesis PAHA, as % of control, in presence of the antibiotic (μg/ml)				
			10 000	1 000	100	10	1
1	Sintomitsin	0.2	28	45	87	93	93
2	»	0.2	13	61	100	—	—
3	»	0.2	0	78	100	—	—
4	Chloramphenicol	0.2	3	19	105	—	—
5	»	0.2	5	66	93	—	—
6	»	0.2	—	67	—	—	—
7	Dextramycetin	0.2	0	42	48	122	—
8	»	0.2	—	55	114	—	—
9	Chlortetracyclin	0.2	—	50	89	89	—
10	»	0.2	—	50	83	—	—

concentrations. The slight inhibition observed at very high concentrations of streptomycin was probably due to the presence of impurities in the antibiotic preparation; a purer preparation of streptomycin, viz., its calcium chloride complex, not only did not inhibit synthesis, but even had a stimulating effect.

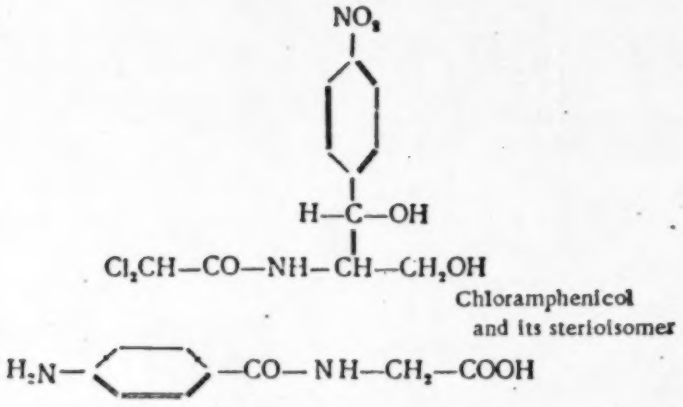
It thus appears that neither the specific action of antituberculosis drugs, nor the toxic effects which they may exert, can be related to their effect on the biosynthesis of hippuric acid and its derivatives.

The widely used antibiotics penicillin, chloramphenicol (levomycetin), syntomycin, and chlortetracyclin, at concentrations such as are achieved in the organism therapeutically, do not inhibit the reaction of biosynthesis of p-aminohippuric acid. Very high concentrations of antibiotics (1000 and 10,000 μg/ml).

It is clear that the mechanism of action of these chemotherapeutic preparations cannot be related to this reaction.

The effect of high concentrations of chloramphenicol, of its d- and dl-forms, and of penicillin and chlortetracyclin on the biosynthesis of PAHA is of theoretical significance insofar as the effect is one of inhibition of a reaction by substances analogous in structure to the products of the inhibited biosynthetic reaction (presence of amide bonds).

As an illustration of the structural similarities we give the formulae of chloramphenicol and PAHA.



Chloramphenicol and its stereoisomer

p-Aminohippuric acid

It should, however, be borne in mind that at such high concentrations these agents may also interfere with other biochemical processes, and this may secondarily affect the process under examination.

The antituberculosis chemotherapeutic preparations Tubazid, Ftivazid, and Tibon, as well as streptomycin, do not interfere with the biosynthesis of p-aminohippuric acid by liver slices.

Penicillin, chloramphenicol, syntomycin, dextramycetin, and chlortetracyclin have no effect on the biosynthetic reaction at concentrations corresponding to therapeutic levels, but at higher concentrations they suppress synthesis, down to total inhibition.

The inhibition of the biosynthetic reaction by chloramphenicol, its stereoisomers, penicillin, and chlortetracyclin may be related to the similarity of molecular structure of the inhibitors and reaction product.

SUMMARY

The effect of chemotherapeutic preparations on the synthesis of peptide bonds was studied.

The reaction of synthesis of para-aminohippuric acid (PAHA) in sections of a rat's liver from para-aminobenzoic acid (PABA) and glycine was used. This method was described by P. P. Cohen and R. W. Gilwery. It was established that antituberculous chemotherapeutic preparation Tubazid, Ftivazid, Tibon, and streptomycin do not disturb biosynthesis of para-aminohippuric acid by liver slices. Penicillin, chloramphenicol,

syntomycin, dextromycetin and chlortetracyclin in low concentrations, which correspond to therapeutic, have no effect on the above reactions of biosynthesis. In high concentrations these drugs suppress this reaction up to complete inhibition. Depression of reaction of biosynthesis of PAHA by chloramphenicol and its stereoisomers, penicillin, and chlortetracyclin may be explained by similarity of the chemical structure of the depressing substances with the products of this reaction.

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PHARMAGOLOGY

EFFECT OF DICHLOROETHANE ON VITAMIN C METABOLISM

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We have shown in a number of published papers [1] that the exercise of the protective function of vitamin C in response to toxic factors is associated with its increased utilization and expenditure in the animal organism. It is of importance that in organisms not capable of the biosynthesis of this vitamin, which has to be supplied with the food, its increased expenditure must lead to impoverishment of the ascorbic acid reserves of the tissues. In organisms able to synthesis ascorbic acid its increased expenditure leads to increased biosynthesis, and its concentration rises in the tissues.

We have examined the effect of the chlorinated hydrocarbon dichloroethane on the ascorbic acid content of the organs of experimental animals capable of its synthesis.

EXPERIMENTAL METHODS

The experimental animals were female albino rats, weighing from 180 to 210 g. The animals were maintained on a mixed diet, with addition of fish oil.

The first series of experiments was on 30 rats, divided into 3 equal groups. They were subjected to a single exposure to dichloroethane, lasting for two hours, in a special chamber. For 2 groups the dichloroethane concentration in the chamber was 20 mg/liter. The first group was killed by decapitation immediately after exposure, and the second was killed a day later. The third group was similarly exposed to a concentration of 10 mg/liter for 2 hours, and the rats were killed immediately after.

The organs were analyzed for ascorbic acid immediately after death, by our usual method [2]. Ascorbic acid was determined in the liver, the walls of the small intestine, the spleen, the brain, the kidneys, the adrenal glands, and the heart.

In the second series of experiments, on two groups of ten rats each, we studied the effects of repeated small doses of dichloroethane, over a long time. One group was exposed daily for two hours to a chamber concentration of 0.3 mg of dichloroethane per liter, and the second to a concentration of 0.6 mg per liter. The animals were killed at the end of a month, during which time they had suffered 25 exposures.

As controls we took a group of 15 rats, which were not subjected to dichloroethane inhalation.

EXPERIMENTAL RESULTS

Experiments on Single Acute Poisoning with Large Doses of Dichloroethane

After a single 2-hour exposure to a concentration of 20 mg of dichloroethane per liter the ascorbic acid content of the liver rose by 80% (T = 8.0) over the controls, and of the spleen by 36% (T = 4.1). A rise of 15% in brain ascorbic acid can be considered to be a very large one, since numerous researches have shown that

brain ascorbic acid varies normally within very narrow limits, and is far less variable than is the ascorbic acid content of the liver, spleen, and certain other organs. The considerable rise in the ascorbic acid concentration of cardiac tissue (30%) is of interest. The range of variation of the ascorbic acid content of the heart is also usually very narrow. The ascorbic acid content of the intestinal wall of the exposed animals was 20% higher than in the controls, but because of considerable individual variations (m = 2.6) the coefficient of significance does not attain the value of 3, although it is not far from it. There was no change in the ascorbic acid content of the adrenals. As for the kidneys, a survey of the results found will be deferred to the section devoted to discussion of results, below.

Statistically significant increases in ascorbic acid content of the organs of rats killed 24 hours after exposure for 2 hours to a concentration of 20 mg of dichloroethane per liter were found for the same organs as the first group (T greater than 3 in all cases), with the difference that the increase in the liver was now only 36%, as compared with 80% in the first group; in the other organs the increase remained the same, and there was still no change in the ascorbic acid content of the adrenals.

Statistically significant, and considerable rises were also found in the last group of rats, exposed to 10 mg of dichloroethane per liter, for the same organs as in the preceding groups, except in the intestine. The ascorbic acid contents of the adrenals and the kidneys remained unchanged.

Experiments on Chronic Poisoning

The ascorbic acid content of all the organs examined was at the normal level in the group of rats which had been exposed 25 times during a month to concentrations of 0.3 mg of dichloroethane per liter.

We were only able to analyze the liver and the brain of the rats of the group similarly given 25 exposures to concentrations of 0.6 mg of dichloroethane per liter. The ascorbic acid contents of these organs were very high, differing so much from the controls that T attained a value of 9.

The large increase in ascorbic acid content encountered in most of the organs of rats which had been subjected to inhalation of dichloroethane is undoubted evidence of a very considerable rise in the rate of synthesis of ascorbic acid. As has been shown earlier [3, 5, 6], such a rise in the rate of synthesis is a compensatory process, in response to an increased rate of expenditure of ascorbic acid, which participates in the defensive reaction of the organism to toxic factors. It hence follows that inhalation of dichloroethane considerably raises the body requirements for ascorbic acid.

We consider that important features of the reaction discovered by us are the selectivity and the dynamics of the changes in ascorbic acid encountered in the various organs, which are characteristic of each toxic factor, in the given case of dichloroethane, under the conditions applied by us. If we consider the results obtained by us from this point of view, we see that an exposure of 2 hours to concentrations of 20 mg of dichloroethane per liter evokes a pronounced response from a barrier organ, such as is the liver, from the central nervous system (the brain), from the blood system (the spleen), and from the myocardium. The rise in ascorbic acid concentration in the walls of the small intestine shows marked individual variations (m = 2.6), so that we can speak of a strong probability that this organ is immediately involved in the defensive reaction of the organism to the action of dichloroethane. We could observe no reaction from the adrenal glands.

The reactions found in the various organs 24 hours after exposure to dichloroethane were of the same kind as immediately after exposure, but were of a different intensity. Thus the intensity of the reaction found in the liver was smaller. An examination of the significance of the difference between the effect of dichloroethane on liver ascorbic acid content immediately after exposure, and 24 hours after, shows that T = 3.6. It is of considerable interest that 24 hours after exposure there is a significant rise in the ascorbic acid content of the walls of the small intestine, with a far smaller range of individual variations (m = 1.4), as compared with the reaction found immediately after exposure. This reaction of the thin intestine suggests the possibility that dichloroethane or its transformation products may be excreted into the intestine.

The reaction of the kidneys is of interest, from this point of view. We could find no significant difference either, immediately after exposure, or 24 hours later, between the ascorbic acid contents of the kidneys of the test animals and of the control group; comparison of the results found immediately after exposure with those found 24 hours revealed, however, a significant difference (T = 3.5). It might hence be supposed that the dynamics of the reaction are such that the maximum change in ascorbic acid concentration takes place at some time

less than 24 hours, for which reason we were unable to detect the corresponding reaction in the kidneys.

The reaction of the blood and central nervous systems, as well as of the myocardium, remained at the same high level at the end of the 24 hour interval.

The reaction found in rats exposed to a concentration of 10 mg of dichloroethane, and killed immediately after, was the same as for the higher dosage level of 20 mg per liter, as regards the organs involved, but the intensity of the reactions was smaller, approaching those found in rats killed 24 hours after exposure to the higher dosage level.

Repeated exposure to low concentrations of dichloroethane (0.3 mg per liter) did not cause any changes in the ascorbic acid content of the organs examined. A very pronounced rise in ascorbic acid content was, however, found in the group of rats subjected to repeated expsoure to concentrations of 0.6 mg of dichloroethane per liter, pointing to the toxic action of this concentration.

A comparison of our findings with published experimental data on the pathological changes produced by dichloroethane affords support to our views.

Thus Heppel, Neal et al., [7] found that rats poisoned with dichloroethane exhibited macroscopically evident hepatic hyperemia, and hyperemia with pin-point hermorrhages in the gastrointestinal mucosa; histological examination revealed degenerative changes in the kidneys, liver, and myocardium. Spenser, Rowe, et al., [8] showed that the most pronounced histological changes were in the kidneys, with smaller ones in the liver and lungs; they also reported biochemical changes in the blood. Larionov [4] found severe degenerative changes in the liver, kidneys, spleen, and heart of mice.

Thus our conclusions, based on a study of the dynamics of changes in distribution of ascorbic acid in various organs of rats poisoned with dichloroethane, are in agreement with published pathomorphological observations and studies of blood composition of rats subjected to the action of dichloroethane.

SUMMARY

The rate of biosynthesis of ascorbic acid and the changes of its concentration in the organs of rats was studied. It was demonstrated that dichloroethane causes increased expenditure of ascorbic acid in the organism of these animals. Dichloroethane, likewise, causes the immediate reaction of the liver, the central nervous system, the blood and the cardiac muscles. Intestines and kidneys respond later. Their reaction is manifested by increased content of ascorbic acid in these organs. This effect is still present 24 hours after a single inhalation of dichloroethane during 2 hours.

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EXPERIMENTAL STUDIES ON DETOXICATION OF MERCURY SALTS BY MEANS OF ETHYLENEDIAMINETETRA-ACETIC ACID

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Ethylenediaminetetraacetic acid (EDTA) reacts with the ions of many heavy metals, forming stable water-soluble complexes, and in this way it inactivates the metals, and facilitates their elimination from the organism. This property has led to its application to the treatment of heavy metal poisoning: lead [6, 13, 14], vanadium [12] (Mitchell, 1953), plutonium [10], manganese (Radier, 1954), and others, and for the inactivation of cobalt [3, 8] (Child, 1951, Belgova, 1956) and cadmium [2], and for acceleration of elimination of radioactive elements from the body, such as yttrium [9, 15].

The object of the present research was to investigate the possibility of inactivating mercury ions by means of EDTA, and to find the necessary quantitative ratios between EDTA and mercury; this problem has not been elucidated in the literature.

For this purpose we selected experimental conditions such that biologically detectable amounts of mercury ion were as small as possible, in order to facilitate the determination of the ratio of EDTA to mercury needed to suppress the toxic action of the latter. A suitable experimental procedure was to prevent development of carbocholine effects on frog heart by means of one of the salts of mercury (mercuric chloride) [1].

The reactivity of an isolated frog's heart to the same concentration of carbocholine (1 : 4,000,000 – 100,000,000) was tested 3 times in each experiment (the carbocholine test).

First carbocholine test. After recording contractions of the heart for 5-10 minutes carbocholine was introduced into the cannula, causing either a reduction of the amplitude of the contractions, or their total arrest. The heart was then washed out with Ringer solution for a minute, to remove carbocholine. Second carbocholine test: after the amplitude of the contractions had returned to the initial level calcium EDTA was introduced into the cannula (concentration, 1 : 10,000 – 20,000), followed after 5-10 minutes by a mercuric chloride solution (1 : 1,100,000); in some experiments mixtures of mercuric chloride and EDTA solutions, of the above concentrations, were made 30-60 minutes earlier, and were added to the perfusion fluid. Reactivity of the heart to carbocholine was again tested (for 1 minute), 10-15 minutes after perfusion with mercuric chloride or mercuric chloride-EDTA mixture. The extent of inactivation of the mercury ion by EDTA was assessed from the intensity of the carbocholine reaction; if it approached that of the first test it followed that the mercuric chloride effect was abolished, indicating binding of mercury ions, while if the carbocholine reaction was weakened or abolished it followed that mercury ions were present.

Third carbocholine test: this was performed after washing out EDTA, mercuric chloride, and carbocholine. Mercuric chloride was perfused, at the same concentration as in the preceding experiment, and was followed after 10-15 minutes by carbocholine. The carbocholine reaction was either absent, or was very weak, in this test. The mercuric chloride reaction was irreversible, and for this reason this test had to be the last of each series. The third test was not performed in many of the experiments, when the second carbocholine reaction was found to be suppressed.

In all, we performed 20 experiments, involving 62 tests.

The molar proportions of EDTA to mercuric chloride were 6, 12, 24, 48, and 120.

EXPERIMENTAL RESULTS

Statistical treatment of the experimental data showed that the effect of mercuric chloride is abolished when the molar ratio of EDTA to mercuric chloride is 120:1. Inactivation of mercuric chloride is not observed when this ratio is lowered to 48:1.

The results found for the isolated heart preparation led us to investigate the possibility of applying EDTA to inactivation of mercury ions in the intact organism. These experiments were performed on 50 adolescent female albino mice, weighing 18-22 g. Mercuric chloride was given by intraperitoneal injection, at a dosage level of 10 μg per g body weight, and calcium EDTA by intravenous injection (dose, 400 μg/g) or subcutaneously (dose, 1 mg/g). The respective molar ratios of EDTA to mercuric chloride were, accordingly, 60:1 and 100:1. At these dosage levels, calcium EDTA alone does not produce any toxic symptoms in mice (10 mice). Injection of mercuric chloride alone, to a control group, caused the death of the animals within 2-3 days, with symptoms of acute apnea (10 mice). Previous administration of EDTA (20-30 minutes before mercuric chloride) did not prevent the death of the animals (30 mice).

DISCUSSION OF RESULTS

The toxic effect of mercuric chloride on an isolated frog's heart was abolished if it had previously been mixed with EDTA, or if the heart had previously been perfused with EDTA. This could only be achieved, however, by the use of a considerable excess of EDTA (120 M EDTA : M $HgCl_2$). Inactivation of the mercury ion could not be achieved in the whole organism by injection of EDTA in approximately the same molar proportions.

Our results indicate that EDTA binds mercury ions much less firmly than it does other metals. Thus we have shown in earlier papers that cadmium [2] and cobalt [3] are inactivated by EDTA at molar ratios of 1 and 2, respectively. It is known, on the other hand, that the toxic effects of mercuric chloride can be abolished by compounds containing sulfhydryl groups, such as mercaptosuccinate [1, 5, 11] and cysteine [4]. It may hence be concluded that mercury ion is much more firmly bound by sulfhydryl groups, for which reason compounds containing such groups are better antidotes to mercury poisoning than is EDTA. This view is supported by the findings of Bell (1955), who studied the effects of EDTA and dimercaprol (BAL) on elimination of mercury and lead in a case of chronic poisoning. EDTA caused a slight fall in excretion of mercury, whereas dimercaprol greatly increased it.

SUMMARY

It was experimentally proved that mercury may be fixated with the aid of ethylendiaminetetraacetic acid. The latter, likewise, promotes excretion of mercury from the body. It was established in experiments on isolated frog's heart that the ratio of the quantity of sublimate and ethylendiaminetetraacetic acid should be 1:120 for total fixation of mercury. However, it was impossible to fixate mercury in the intact organism, even in presence of this ratio.

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MICROBIOLOGY AND IMMUNITY

ON THE PARTICIPATION OF THE RENAL TUBULE IN THE MECHANISM OF EXCRETION OF DYSENTERY ANTIGENS BY THE KIDNEY

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The fact that the kidneys excrete many large molecular substances (serum proteins, dextrins, complete antigens, bacteria of the enteric group and others) can be regarded as established at the present time [3, 13, 16, 18, 19, 20].

Terry, Sandrock, Hye and Whipple [20] and Addis [11] suggest that plasma proteins pass through the glomerular membrane and when the function of the renal tubule is normal are completely reabsorbed in its proximal regions. Under conditions of increased plasma protein concentration (10-11%), a larger amount of protein, having passed the glomerular membrane, is not completely reabsorbed, and a significant portion of it appears in the urine. The amount of protein in the glomerular filtrate of a man is estimated at 50 g per day, and the concentration in the filtrate as 0.03 [3, 18].

There are data on the influence of the immunological state of the body on the excretion of many antigens and viruses [1, 4, 5, 8, 10].

N. B. Yafarova [10] suggested a role of the tubules in the excretion of dysentery antigens by the rabbit, considering that it is secreted by the epithelium of the renal tubules.

In the present study we attacked the following problems: 1) to estimate the excretion of dysentery antigens by the kidneys of the dog, 2) to elucidate the influence of changes in diuresis on the extent of excretion of dysentery antigen, 3) to study the influence of maximum reabsorption of glucose on the excretion of dysentery antigen, 4) to elucidate the influence of dysentery antigen on the reabsorption of glucose, 5) to determine the influence of change in the immunological state of dogs on the processes just mentioned.

EXPERIMENTAL METHODS

Dogs weighing 8 to 18 kg were used as the experimental animals. The investigations were made during a prolonged experiment on 16 dogs. About an hour before the start of administration of the antigen the dogs were given a water load of 40-45 ml per kg body weight. Before administration of the antigen blood and urine samples were taken. We administered the antigen in the amount of 0.1-0.4 mg per kg of body weight in a solution of 500-800 ml sodium thiosulfate or glucose. The substance under investigation was given by intravenous drip during the course of each experiment. The experiment consisted of 5-6 clearance periods of 10-20 minutes each. The bladder was emptied by catheterization or the urine was collected through a Pavlov-Orbeli fistula. In the middle of the clearing period blood was taken.

The concentration of thiosulfate in the blood and urine was determined iodometrically, and the amount of antigen in the blood and urine was determined by complement fixation in the cold.

The immune serum used in the reactions was a polyvalent precipitating anti-dysentery serum with a titer of 1:800,000, obtained from the Moscow Municipal Bacteriological Institute; it had a complement-fixing titer

of 1:2,000,000. Using this serum, it would have been possible to detect in 1 ml of the fluid being investigated 0.0000005 g or 0.0005 mg of antigen. The amount of glomerular reabsorption of glucose was calculated from the formula

$$Rg = Pg.\,Ot - Mg.\,D\ (mg/min.)$$

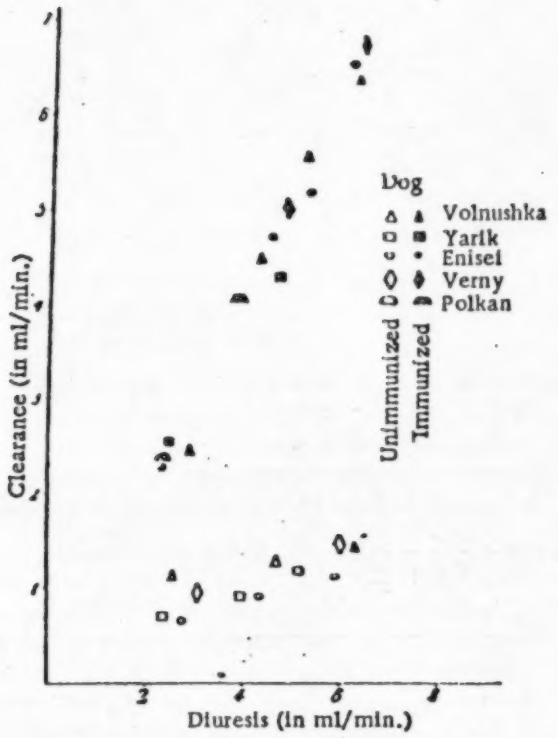
where Rg is the reabsorption of glucose, Pg the plasma glucose in mg%, Ot the thiosulfate clearance, Mg the urine glucose in mg%, and D minutes of diuresis.

The investigations began with the excretion of dysentery antigens by 9 dogs which had not been immunized, and 6 dogs immunized with dysentery vaccine. The results of the experiment are presented in Table 1, and agree with the data from the investigations of N. B. Yafarova, A. A. Polner and I. M. Khakberdyev (1956).

In the following series of experiments, with the aim of elucidating the role of the glomerular capsule and the renal tubule in the process of excretion of dysentery antigen by the kidney, we carried out experiments with different water loads, i.e., the infusion of 300 to 800 ml of sodium thiosulfate solution along with the antigen.

EXPERIMENTAL RESULTS

The results of the experiments are shown in the accompanying figure.



Effect of change in diuresis on antigen clearance.

As is seen from the figure, increased diuresis in the unimmunized dogs produced a negligible increase in excretion of the antigen. When diuresis was 2.3-2.75 ml/min. the excretion of antigen per minute was 0.003-0.005 mg; when diuresis was increased to 5-6 ml/min. the amount of antigen excreted was 0.005-0.006 mg/min. What explains such an insignificant increase of antigen excretion when diuresis is increased 2-3 times? Experiments showed that increase of diuresis in the unimmunized dogs was accompanied by a decrease in the concentration of the indicator substance. With diuresis of 2.3-2.5 ml/min., the concentration of the indicator substance reached 0.5, with diuresis increased to 5-6 ml/min. the concentration of the indicator fell in 2 cases to 0.25. In the immunized dogs increase in diuresis exhibited a positive effect on the elimination of dysentery antigens by the body. This was confirmed by repeated experiments on the same animals (Volnushka, Polkan, Verny, Enisei, Yarik; see Figure).

During relatively low diuresis (2.25-3.5 ml/min.) and with indicator concentrations equal to unity, clearance of antigen varied within range 2.43-4.5 ml/min., and under conditions of increased diuresis (4-6.6 ml/min) amounted to 4-6.6 ml/min., (see Figure). The concentrations of the indicator, in spite of considerable increases in diuresis, did not decrease.

It must be pointed out that raising the water load increase of diuresis are not accompanied by any significant change in the glomerular filtrate. From this it may be conjectured that the increase in excretion of antigen, observed when the water load is raised, is connected first of all with the activity of the tubules. This conjecture is supported by the fact of considerable activitation of antigen excretion by increased diuresis, especially in immunized animals (see Figure).

The influence of maximum secretion of para-aminohippuric acid of maximum reabsorption of glucose on reabsorption of ascorbic acid was demonstrated by the investigations of Selkurt, 1944.

Pitts, 1944, established a blocking of the reabsorption of ceratin by increased reabsorption of glycine.

TABLE 1
Excretion of Antigens by Dog Kidneys

No. p/p	Dog's name	Date of experiment (1955)	Antigen					
			Concentration in plasma (mg%)	Concentration in urine (mg%)	Indicator concentration	Glomerular filtration of thiosulfate	Diuresis per minute (in ml)	Antigen clearance (ml/min.)
			Unimmunized					
1	Volushka	5/V	1.4	0.2	0.5	188.2	2.5	1.25
2	Enisei	6/V	0.4	0.1	0.25	209.0	2.75	0.68
3	Yarik	30/V	0.2	0.1	0.5	187.6	2.3	0.75
4	Kudrash	6/V	0.4	—	—	188.3	7.0	—
5	Verny	11/V	0.4	0.1	0.25	218.4	6.0	1.5
6	Saturn	25/V	0.4	0.1	0.25	210.0	5.0	1.25
7	Zhuchka	31/V	0.1	—	—	206.0	2.0	—
8	Jack	30/III	0.1	—	—	206.0	2.8	—
9	Ace	31/V	0.1	—	—	206.0	2.0	—
			Immunized					
1	Kudrash	13/X	0.8	0.8	1	125.2	3.9	3.9
2	Polkan	17/V	0.8	0.8	1	140.0	4.0	4.0
3	Martyshka	28/IX	0.8	0.8	1	116.0	4.75	4.75
4	Volnushka	15/IX	0.4	0.4	1	139.5	4.5	4.5
5	"	27/IX	0.4	0.8	2	136.9	3.4	6.8
6	"	17/X	0.4	0.8	2	115.15	2.45	4.9
7	Verny	5/VII	0.8	0.8	1	164.3	6.6	6.6
8	"	15/VII	0.8	0.8	1	132.4	6.5	6.5
9	Yarik	1/IX	0.4	0.4	1	123.5	2.25	2.25
10	"	14/X	0.4	0.4	1	126.0	2.5	2.5

On the basis of these investigations one may suggest the possibility of a reciprocal effect of antigen and glucose on their excretion by the kidneys. With the object of elucidating this we carried out the experiments of the following series. It is well known that glucose in concentrations below its threshold value is completely reabsorbed and not excreted in the urine. In otder to make possible an estimate of the speed of excretion we maintained concentrations of glucose in the blood of the order of 400-463 mg%.

We carried out the experiments on 6 unimmunized and 6 immunized dogs. The studies carried out to investigate the effect of antigen on the reabsorption of glucose are presented in Table 2. From Table 2 it is evident that in unimmunized dogs the excretion of glucose in the urine amounted to 401.4-406.3 mg/min., while the reabsorption varied from 347.8 to 385.1 mg/min., i.e., somewhat exceeded the maximum reabsorption of glucose established by Shannon, Farber and Troast (1941).

Evidently antigen, when first introduced into the body, stimulates the reabsorbing system of the kidney and exalts the processes of absorption. In immunized dogs, under the influence of dysentery antigens the maximum reabsorption of glucose declined about 30-33% on the basis of the data of Shannon, and amounted to 162.8-247.6 mg/min.

Study of the influence of maximum reabsorption of glucose on the excretion of dysentery antigens by the kidney revealed several peculatities of the process of clearing the body of antigen.

Excretion of dysentery antigens by the kidneys of unimmunized dogs under conditions of maximum reabsorption of glucose was accomplished at a rate 3-4 times that in control experiments and amounted to 0.017-0.018 ml/min. The concentration index of the antigen, in spite of the 2-2½ fold increase in diuresis, did not decrease. Antigen clearance reached 4.3-4.5 ml/min., exceeding that in control experiments 3-4 times (see Table 2). Our experiments showed that maximum reabsorption of glucose increases the excretion of antigen by the kidneys of unimmunized dogs 3-4 times. In dogs immunized with dysentery vaccine the excretion of antigen by the kidneys during maximum reabsorption of glucose reached 0.075-0.096 ml/min. and was increased 2-3 times in comparison with control experiments.

In spite of the elevated diuresis, leading to glycosuria, the concentration index of the dysentery antigen remained equal to unity. Antigen clearance varied from 9.4 to 12.9 ml/min.

On the basis of study of the results of the experiments of the series we suggest that maximum reabsorption of glucose, producing an overload of sugar, promotes increased excretion of antigen by the kidneys of unimmunized and immunized dogs.

Investigation of the variations in filtration by the clearing coefficient of sodium thiosulfate reveals some depression. It might be suggested that complicated phenomena of reabsorption and secretion of antigen take place simultaneously in the glomerular apparatus of the kidneys, similar for example to the excretion of urea. If a determining importance of reabsorption is recognized, then we should admit the possibility of significant filtration of antigen through the capsular membrane.

However, according to the available data of Wallenius (1954) and Giebish, Lauson and Pitts (1954) filtration of the macromolecular fractions is inversely proportional to the molecular weight. The proteins of human blood plasma, having molecular weights from 35,000 to 170,000, appear in the filtrate up to 0.03%. It may be supposed that the dysentery antigen, consisting of various fractions with molecular weights from 10^6 to 10^4 and less, behaves in the blood as a substance consisting of various amounts of fractions with the above mentioned high molecular weights. If we assume that 1/10 of the total antigen present in the blood falls into the fraction having molecular weights of 10^5 and less, then the amount of antigen appearing the glomerular filtrate amounts to about 1/2000 of that found in the plasms (Webster, Sagin, Anderson, Breese, Freeman and Landy, 1953).

On this assumption, as is seen from Table 2, the amount of antigen filtering through in one minute is several times smaller than the amount excreted in the final urine in the same time. Under the stated condition of antigen filtration, in unimmunized dogs the amount of antigen excreted in the final urine is 13.2 times the amount in the filtrate. In immunized dogs the ratio of the excreted and filtered antigen rises to 61.9.

From this there arises the necessity of admitting the existence of a second mechanism of secretion of antigen, i.e., its secretion by the epithelium of the renal tubules, similar to the excretion by the kidney of colloidal dyes and molecularly dispersed substances (phenol red, uric acid, phosphate, penicillin, para-aminohippuric acid, and others).

TABLE 2

Influence of Maximum Reabsorption of Glucose on Excretion of Dysentery Antigens by Kidneys of the Dog

No. p/p	Name of dog	One minute's urine in ml	Thiosulfate clearance (in ml/min.)	Antigen							Glucose				
				concentration in plasma (in mg %)	concentration in urine (in mg %)	concentration index	filtration (in mg/min.)	excretion of antigen in urine (in mg/min.)	relation of excreted antigen to that filtered off	antigen clearance (in ml/min.)	concentration (in mg % in plasma)	concentration in urine (in mg %)	filtration (in mg/min.)	excretion in the urine (in mg/min.)	reabsorption (in mg/min.)
1	Volushka	2.5	188.2	0.4	0.2	0.5	0.0003764	0.005	13.2	1.25	—	—	—	—	—
2	Enisei	2.25	209.0	0.4	0.1	0.25	0.000418	0.00225	6.5	0.68	—	—	—	—	—
3	Yarik	2.3	187.6	0.2	0.1	0.5	0.0001876	0.0023	12.2	0.75	—	—	—	—	—
4	Kaiya	8.9	192.5	0.4	0.2	0.5	0.000385	0.0178	46.2	4.45	404	4 565	777.7	406.3	371.4
5	Lada	9.0	185.5	0.4	0.2	0.5	0.000371	0.018	48.5	4.5	424	4 460	786.5	401.4	385.1
6	Spark	8.6	164.0	0.4	0.2	0.5	0.000328	0.0172	52 4	4.3	463	4 690	251.1	403.3	347.8
7	Kydryash	3.9	125.2	0.8	0.8	1	0.0005008	0.031	61.9	3.9	—	—	—	—	—
8	Polkan	4.0	140.0	0.8	0.8	1	0.00051	0.032	62.7	4.0	—	—	—	—	—
9	Martyshka	4.75	116.0	0.8	0.8	—	0.000464	0.038	81.8	4.75	—	—	—	—	—
10	Kudryash	9.4	120.0	0.8	0.8	1	0.00048	0.0752	156.6	9.4	400	2 640	480	258.7	231.3
11	Lisa	12.8	118.0	0.8	0.8	1	0.000472	0.0924	195.7	12.8	405	2 540	477.9	325.1	152.8
12	Renka	12.0	128.0	0.8	0.8	1	0.000512	0.096	187.8	12.0	406	2 200	511.6	264.0	247.6
13	Martyshka	12.9	120.0	0.8	0.8	1	0.00048	0.0932	194.1	12.9	422	2 470	506.4	308.6	197.5

Secretion of antigen by the cells of the renal tubules of unimmunized and immunized animals is significantly different. Before immunization secretion exceeded filtration of antigen 10-13 times, in immunized dogs secretion was 50-60 times filtration.

The phenomenon of depression of maximum reabsorption of glucose under the influence of dysentery antigen, found by us, may be regarded as a partial blocking of tubular reabsorption of glucose, brought about by an increase in secretion of dysentery antigens by the epithelium of the renal tubules, similar to the relations found, for example, in studying the influence of secretion of para-aminohippuric acid on the reabsorption of ascorbic acid (Selkurt and others).

SUMMARY

In 56% of cases primary introduction of dysentery antigen to unimmunized dogs was accompanied by slight excretion of antigens. Excretion of antigens by kidneys after immunization was accelerated by 5-6 times. Increased diuresis in immunized dogs increased antigen excretion correspondingly. Prior to immunization, accelerated diuresis decreased the concentration index, the antigen excretion not being pronounced. Glucose loading, instituted to maximal reabsorption, increased dysentery antigen excretion. The dysentery antigen depressed the maximal reabsorption of glucose in immunized dogs. A certain relation in connection with increased dysentery antigen excretion by kidneys in the process of immunization may be attributed to functional changes of cells of the glomerular apparatus.

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EFFECT OF CERTAIN ANTIBIOTICS ON ACTIVE IMMUNITY IN WHITE MICE

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The question of the influence of antibiotics on the immunological processes of the body acquires a greater significance in connection with the wide use of these preparations in the treatment of a whole series of infectious diseases.

The extensive literature devoted to the study of the influence of antibiotics on the production of antibodies hardly touches on the question of the effect of antibiotics on the survival of vaccinated animals infected with a virulent strain of a pathogen infectious for them.

Having established in previous work [1, 3] that prolonged administration of a number of antibiotics acts unfavorably on the production and circulation in the blood of agglutinins, we decided to investigate whether this demonstrates an influence of these substances on the production of active immunity in white mice. With this aim in mind we selected penicillin, streptomycin, syntomycin and its levo- and dextrorotatory components, levomycetin and dextromycetin.

EXPERIMENTAL METHODS

We divided mice weighing 18-20 g into 3 groups in each experiment. The mice of the first (experimental) group were vaccinated by three injections of killed typhoid vaccine, obtained from the N. F. Gamaleya Institute, in doses of 100, 200, and 200 million microbes at intervals of 7 days. The mice also received at the same time as the vaccine one of the antibiotics being studied.

The mice of the second (control) group were vaccinated only.

To the third (control) group belonged the mice receiving only the antibiotic being investigated (without vaccination). This control group was designed to reveal whether merely the injection of the antibiotic might produce some effect on the survival of the mice after infection.

We administered the syntomycin, levomycetin and dextromycetin to mice by mouth in 0.5 ml of a 1% starch solution from a special dispenser, twice a day daily, in the amount of 4 mg of the material per mouse.

The vaccinated control mice received only pure starch. We administered the penicillin and streptomycin in distilled water in the amount of 1000 units per mouse. At the end of the course of vaccination the mice received the preparation under investigation in the course of a further 10 days, but 2 days before infection the administration of the antibiotics was stopped, and the mice infected by intraperitoneal injection of typhoid culture.

In order to make sure that at the time of infection the bodies of the mice did not contain antibiotics in concentrations sufficient to protect them from the action of the injected microbes, we determined the presence of these substances in the liver and blood serum of the mice.

TABLE 1

Effect of Antibiotics on Microbial Growth

Antibiotic	Serum dilutions 1	2	3	4	5	6	7	8	9	10	11	12	Liver dilutions 1	2	3	4	5	6	7	8	9	10	11	12	Standard concentrations (in gamma) 10	5	2.5	1.25	0.62	0.31	0.15	0.07	0.04	0.02	0.01	0.005
Syntomycin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+											
Levomycetin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+											
Dextromycin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+											
Penicillin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+											
Streptomycin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+											

We ground up the liver in a sterile mortar with quartz sand and physiological saline in the proportions of 1 ml saline to 1 g liver. We centrifuged the emulsion, transferred 0.2 ml of the supernatant fluid to a test tube containing 0.2 ml of Hiss' medium with glucose infected with the test organism (Staphylococcus in titrating penicillin and enteric bacilli in titrating streptomycin and syntomycin). We inoculated the Hiss medium by adding 250,000 bacilli to 1 ml. We carried out the titrations by the method of serial dilutions.

Serum of blood taken from the mice by cardiac puncture was titrated for the property mentioned above. As a control we took the antibiotic under investigation in the amount of 10 γ of the substance for the initial dilution. In all 12 dilutions were made, starting with a 1:2. We placed the test tubes in the thermostat for 18 hours, then determined the presence or absence of the antibiotic by the change in color of the Hiss medium which attested growth of the microbes.

EXPERIMENTAL RESULTS

As is seen from Table 1, about 24 hours after completion of the administration of the antibiotics they are already not demonstrable in the liver and serum.

Thus we were able to infect the experimental and control animals without fear that the chemotherapeutic preparation was still circulating in their bodies.

The principal requirement, without which it would not be possible to set up experiments to detect active immunity, is the availability of a pathogen, in particular typhoid, of a strain of sufficiently high virulence. But even when infecting with strains of high virulence, the numbers of microorganisms required to bring about the death of the experimental animal sometimes reach very high figures (hundreds of millions). It must accordingly be admitted that the death of mice, when such colossal doses of culture are employed, depends to a significant degree upon the intoxication produced by the typhoid endotoxin which is liberated when the microorganisms disintegrate [6].

This fact explains the desire to increase the virulence of the microorganisms so as to reduce as much as possible the amount of them introduced.

In our method of infecting mice we used a young broth culture. We used a 4-hour broth culture, since according to the findings of Craigie and Brandon [4] Khristova-Baldina [2], and others this culture contains the largest proportion of V-forms, i.e., forms with the high virulence; in addition, we added to the broth 0.5% glucose, which stimulated the development of the bacteria and promoted the accumulation of the Vi antigen [5].

TABLE 2

Survival of Mice Following Treatment with Antibiotics

Group of animals	Number of mice in experiment	Number of mice surviving (actual numbers)
1. Experiment Vaccinated mice receiving antibiotic		
Syntomycin	100	50
Levomycetin	100	70
Dextromy	90	9
Penicillin	90	9
Streptomycin	80	16
2. Experiment Vaccinated mice	100	70
3. Control Mice receiving only		
Syntomycin	25	0
Levomycetin	25	1
Dextromycetin	25	0
Penicillin	23	0
Streptomycin	25	0

To infect the mice in a conclusive experiment we employed strain 4446, the MLD of which was 0.125 ml of a 4-hour broth culture, which contained approximately 120 million microorganisms. After infection the mice were kept under observation for 5 days.

In the results of the experiments we carried out, the following was obtained (Table 2). Mice which had received only antibiotic succumbed after infection in 10% of the cases during the 5 day period, i.e., none of the antibiotics used showed any protective action against the infection which followed. Thirty percent of the control mice, merely vaccinated with typhoid vaccine and not receiving antibiotics, succumbed; the same per cent of the mice receiving levomycetin succumbed, as of the controls, but with the difference that death occured somewhat earlier in this group, i.e., the mice died after about 24 hours, while the controls died in the course of 3 days.

In the experiment using syntomycin 50% of the mice died, and in the group receiving dextromycin, 90%.

Thus on the basis of these data it may be suggested that prolonged application of levomycetin does not exert any important influence on the building up of immunity in white mice.

The application of syntomycin, and especially dextromycetin, hinders the development of immunity. These results make possible the suggestion that the negative influence of syntomycin depends on its levorotatory inactive component. In experiments in which penicillin was used 90% of the animals succumbed, and in experiments in which streptomycin was used, 80%, which clearly demonstrates the inhibitory action of these preparations on immunogenesis in experimental animals.

SUMMARY

Certain antibiotics exert a negative influence on the production of active immunity by white mice immunized with killed typhoid vaccine. During the course of immunization various antibiotics were administered and mortalities of the various groups of mice, following experimental infection, recorded. If syntomycin were given 50% of the mice died, if dextromycin, 90%, penicillin, 90%, streptomycin 80%; 30% of the control group

and the group receiving levomycetin died. However the latter succumbed in 24 hours, while those in the control group died after 3 days.

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ACTION OF NOVOEMBIKHINE* ON COMPLEMENT AND PRODUCTION OF AGGLUTININS AND PRECIPITINS IN ANIMALS

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Preparations of the chlorethylamine group (embikhine* and others) are employed in the treatment of proliferative diseases of the hematopoietic system (lymphogranulomatosis, leukosis) and with some malignant tumors. In small doses the chlorethylamines may be employed in nonhyperplastic diseases, for example in bronchial asthma [2], and in other internal, infectious and nervous diseases [1]. Obviously the mechanism of action of chlorethylamines is complicated and varied. The suggestion has been made that chlorethylamines in small doses may exert a stimulating action on the protective and compensatory reaction of the body [3]. However, this aspect of the action of chlorethylamines has been studied very little.

The purpose of the work presented here was to investigate the action of the parent compound of a group of chlorethylamines, novoembikhine [4], on complement and the production of agglutinins and recipitins.

The data on this question in the literature apply only to large doses and boil down to this:

Watkins and Wormall [7, 8] showed that embikhine in large dose (0.5-2.8 mg/kg) exerts an inactivating action on complement. Bukants, Dammin, Wilson, Johnson and Alexander [5] established that periodic intravenous injection of embikhine in large dose inhibits the production of antibodies, as well as the development of the vascular disease of rabbits which was produced by the intravenous injection of a massive dose of horse serum. Spurr [6] came to the conclusion that "a single preliminary injection of embikhine (0.5-1 mg/kg) into rabbits represses the production of antibodies in response to typhoid antigen."

EXPERIMENTAL RESULTS

Influence of novoembikhine. Experiments on normal animals interested us especially, as contrary to the study of this question in the clinic, in normal animals there is excluded the influence of pathological processes, which themselves depress the complement function of the serum in nearly all diseases.

Our preliminary experiments in vivo were carried out on rabbits to which, in the course of other experiments we administered novoembikhine about 1-10 days before determining the titer of complement. Further experiments were carried out with the complement of 25 rabbits, to which we administered, about 24 and 48 hours, about 4 and 10 days before, our usual dose of (1.5 mg/kg). In these experiments the complement titer was higher in a significant portion of the rabbits than in the controls.

After this a special experiment was set up with 5 rabbits, whose complement titer was determined three times during the 9 days before the administration of novoembikhine and four times afterward (Fig. 1).

From Figure 1 it is seen that the absolute complement titers before the administration of embikhine in the first three experiments varied significantly (from 0.16 to 0.08 ml), but in spite of this, in all 5 experiments

* Transliteration of Russian – Probably Russian trade name – Publisher's note.

they were more often average (0.1-0.12 ml) or lower (0.14-0.16). After the administration of novoembikhine the absolute titers of complement in all 5 experiments were more stable and not so high, namely in the range 0.06-0.08 ml (see the first three titrations). Experiment No. 1 (Rabbit No. 961) is an exception, where a high titer of complement in the first three titrations (0.02-0.04) fell to 0.16 in the third titration, which is possibly connected with some special moment.

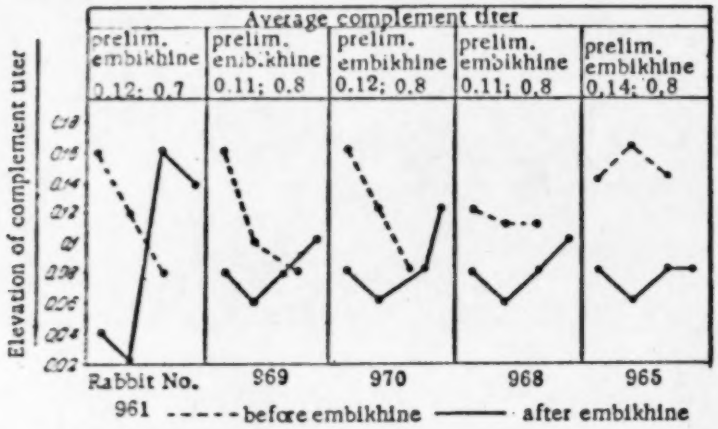

Fig. 1. Effect of novoembikhine on complement liter in rabbits (in vivo).

In all experiments the initial dilution of complement for the titrations was a 1:1 dilution of rabbit serum in physiological saline. The titration of complement was carried out in a volume of 1 ml according to the usual scheme. We added complement to a series of test tubes in steps of 0.02, that is 0.02, 0.04, 0.06, 0.08, etc.; hemolysin in triple titer, erythrocytes 1:40. The complement titer was determined 30 minutes after 30 minutes exposure in the thermostat to 37°.

Effect of novoembikhine on the production of agglutinins. Experiments were set up on 48 chinchilla rabbits weighing 2.5 and 3 kg. In the majority of experiments we administered the novoembikhine in a single dose of 1.5 mg/kg (intravenously). Each series of experiments consisted of three groups of animals: into one group we injected novoembikhine and antigen simultaneously; into the second, first we injected novoembikhine and after 4 days, antigen; into the third, first antigen, then after about 4 days novoembikhine.

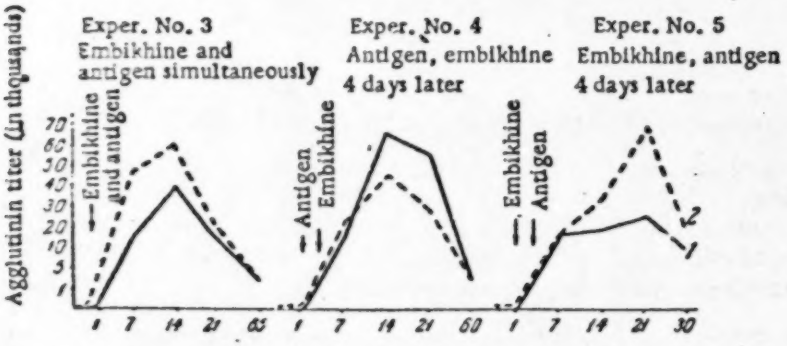

Fig. 2. Influence of embikhine on the production of agglutinins in variously arranged experiments.
1) experiment, 2) control.

For the production of agglutinins we used typhoid diagnostic antigen, injected once subcutaneously, and to determine the resulting agglutinins we added 0.1 ml in each test tube to the corresponding dilution of serum. The agglutination reaction was carried out in a volume of 1 ml. The reaction was estimated according to the characteristics of the precipitate at the bottom of the test tube, without shaking after the racks had been for two hours in the incubator and then up to an hour in the refrigerator.

In each experiment there were 5 experimental and 4 control rabbits. The results of the experiments are presented in Figure 2.

From Figure 2 it is seen that on injecting antigen (typhoid diagnostic antigen)simultaneously with novoembikhine or about 4 days after the injection of novoembikhine there is observed an inhibition of the production of agglutinins for the administered antigen. The interval of 4 days between injection of novoembikhine and antigen was chosen because in that time (on the 4th day) there is generally observed the maximum depression of hematopoiesis, shown by a decrease in the number of leukocytes.

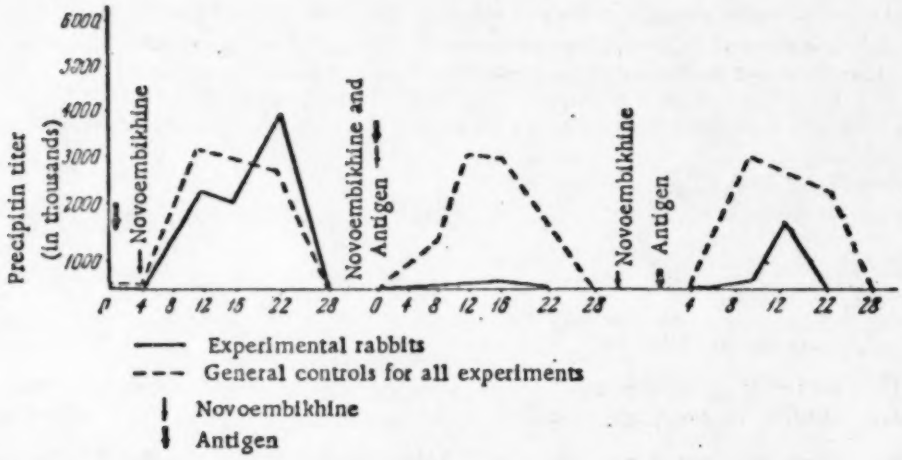

Fig. 3. Influence of novoembikhine the production of precipitins following injection of single doses of novoembikhine, 1.5 mg/kg rabbit weight, and immunization with horse serum (2 injections), 1.5 ml per rabbit. On the left-antigen, after 4 days novoembikhine; center, novoembikhine and antigen simultaneously on the right, novoembikhine, after 4 days antigen.

We obtained different results in the other animals into which we first injected antigen and then (also 4 days later) novoembikhine. In these rabbits the agglutinin titer on the 14th and 21st day after injection of antigen was higher than in rabbits to which we gave no novoembikhine.

In two other experiments in which we injected antigen at weekly intervals, on the 14th day there was also observed some rise in antibody titer in the experimental animals, compared with the titer of the controls.

Influence of novoembikhine on the production of precipitins. To produce precipitins we injected rabbits with unpreserved horse serum as antigen.

For the determination of precipitins we used the ring precipitin reaction. We heated the experimental sera to 56° for 3 minutes and diluted them 1:2 with physiological saline. Readings were made after incubating 30 minutes at 37°.

The results of the experiments are presented in Fig. 2, from which it can be seen that following simultaneous injection of antigen and novoembikhine there is observed a very sharp inhibition of antibody production; following injection of antigen about 4 days after novoembikhine there was noted an inhibition of precipitin

production, but it was definitely weaker. In an experiment in which novoembikhine was injected about 4 days after the antigen, after a period of some inhibition of precipitin production, on the 22nd day the precipitin titer was higher than in the controls.

The data obtained confirm that when novoembikhine is injected after the antigen there is observed a temporary repression, then an increase in antibody production, consequently under certain conditions novoembikhine may exert a stimulating action of the protective reaction of the body. These data, perhaps, may possess practical interest for the production of immune sera, although for this, supplementary experiments are necessary. Inhibition of antibody production, obtained at first by simultaneous injection of antigen some days after administration of novoembikhine, evidently, is explained by a general inhibition of body function for a few days after administration of the preparation.

SUMMARY

The effect of novoembikhine, i.e., di-(2-chlorethyl)-2-chlorpropylamine, on the complement function of the serum as well as on production of agglutinins and precipitins was studied. Experiments were performed on healthy rabbits. Novoembikhine was injected intravenously, 1.5 mg per kilogram of body weight, usually in a single dose. It was established that within 9 days following the introduction of novoembikhine there is an increase and stabilization of the titer of the complement. This was compared with its variations and activity in the same animals previous to introduction of this preparation. Depending on conditions of experiment novoembikhine either inhibited or stimulated the production of agglutinins and precipitins.

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ONCOLOGY

CHANGES IN THE ANTIGENIC PROPERTIES OF TUMOR CELLS UNDER THE EFFECT OF X-RAYS IN VITRO

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The question of the effect of ionizing radiation on the animal organism has been studied by many investigators.

L. F. Larionov [5] has presented data on the biochemical changes of desoxyribonucleoproteins under the effect of penetrating radiation; S. N. Aleksandrov [1] and N. V. Eltsina [2] observed changes in the rate of protein synthesis and energy exchanges of tumor cells under the effect of x-rays; R. Abrams [7] B. Holmes and L. Mee [8] studied changes in protein metabolism of the animal organism exposed to ionizing radiation.

N. N. Zhukov-Verezhnikov, I. N. Maisky and V. S. Gostev [3] put forward a suggestion concerning the possibility of the appearance of new antigenic properties on subjection of tumor cells to penetrating radiation. R. V. Petrov and L. I. Ilyina [6] observed changes in the antigenic properties of hepatic tissue elements and intestinal mucosa of white rats exposed to total roentgen irradiation. L. A. Zilber, V. A. Artamonova, G. M. Frank and A. D. Snezhko [4] report changes in the antigen composition of liver and spleen cells in rabbits following ionizing irradiation.

The present work is concerned with the problem of whether ionizing radiation exerts any effect on the antigen properties of tumor cells.

EXPERIMENTAL METHODS

Experiments were carried out on Ehrlich's ascitic tumor cells (adenocarcinoma) irradiated in vitro with x-rays (dose 5000 r).

The freshly obtained ascitic cells were washed free of ascitic fluid with physiologic solution (three washings), being each time spun in a centrifuge at 2000 rpm. The washed cells were placed in small agglutination test tubes of equal diameter which had been previously weighed. The weight of the ascitic cells in the test tubes was so adjusted that on addition of 2 ml physiologic solution, 0.4 ml of the obtained suspension contained, according to experimental conditions, 2, 6, 10, 20 mg etc. of ascitic cells. The test tubes with the tumor cells were stoppered tightly with rubber bungs and shaken. Half the prepared test tubes was subjected to x-ray irradiation for 10 minutes (current 10 mA, voltage 180kV, no filter, dose 500 r per minute).* The remaining test tubes served as controls. The viability of the irradiated cells was tested by subcutaneous inoculation of white mice.

Changes in the antigenic properties of the cells under the effect of x-rays were studied by means of the anaphylactic reaction with desensitization, sensitization being effected subcutaneously and desensitization and the resolving administration – intracardially.

The interval between sensitization and desensitization was 22-25 days; the intervals between repeated intracardiac injections were 2 hours. Guinea pigs sensitized with irradiated Ehrlich's ascitic cells were

* The irradiations were performed at the Radiobiologic Laboratory of the Institute of Normal and Pathologic Physiology, AMN SSSR, with consultant help of the Director, N. N. Lebedev.

desensitized with non-irradiated ascitic cells. After checking for complete desensitization, irradiated Ehrlich's ascitic cells were administered as the resolving element.

Guinea-pigs sensitized and desensitized with non-irradiated ascitic cells served as controls, as did guinea-pigs which were used for testing the toxicity of the doses of preparations to be used for resolving.

EXPERIMENTAL RESULTS

As can be seen from the Table, guinea pigs sensitized with irradiated Ehrlich's ascitic cells did not show complete desensitization on administration of non-irradiated ascitic cells.

TABLE 1

Results of Active Anaphylaxis Reaction with Desensitization with Irradiated and Non-Irradiated Ehrlich's Ascitic Cells

Sensitization. Antigen—irradiated ascitic cells	Desensitization. Antigen — native ascitic cells								Resolving administration. Antigen irradiated ascitic cells	
	1st administration		2nd administration		3rd administration		4th administration			
dose (in mg)	dose (in mg)	reaction	dose (in mg)	reaction	dose (in mg)	reaction	dose (in mg)	reaction	dose (in mg)	reaction
10	2	±	6	++	10	—			10	+
10	2	+	6	+++	10	—			10	+
10	2	++	6	±	10	—			10	++
10	2	+	6	++	10	—			10	+++
20	6	+++	10	+	20	—			20	+++
20	6	++	10	±	20	—			20	++
20	6	++	10	—	20	—			20	+
20	6	+++	10	++	20	—			20	++
50	2.5	±	7.5	±	25	—	50	—	50	+
50	2.5	++	7.5	+	25	±	50	—	50	++
50	2.5	+	7.5	++	25	—	50	—	50	+++

Note: The guinea pigs which were used for testing the toxicity of the preparations used were not included in the Table since administration of irradiated and untreated tumor cells in doses equal or even exceeding those used in resolving administrations did not elicit any discernible reaction in the animals.

Signs: — absence of reaction; ± occasional scratching of face and sneezing; + repeated scratching of face and sneezing; ++ the same plus coughing, unsteadiness; +++ the same plus convulsions, urinary and fecal incontinence.

This suggests the conclusion that changes occur in the antigenic structure of Ehrlich's tumor cells under the effect of ionizing radiation (in this case roentgen rays). New antigenic properties, absent in non-irradiated ascitic cells, would appear to arise under these conditions.

SUMMARY

Complete desensitization of guinea pigs did not occur, if Ehrlich's ascitic cells, not irradiated by x-rays, were introduced following sensitization of these animals by the ascitic cells, previously irradiated by x-rays (the dose being 5000 r). Evidently, there is a change in the antigenic structure of Ehrlich's tumor cells, which takes place under the effect of x-rays.

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EXPERIMENTAL BIOLOGY

INVESTIGATION OF PHYSIOLOGIC REGENERATION IN THE RAT SMALL INTESTINE FOLLOWING ITS PARTIAL RESECTION

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Following resection of part of the intestine definite morphologic changes take place in the remaining part, viz. there is enlargement of the lumen, thickening of the mucosa and the annular muscular layer, hypertrophy of the lymphatic elements, increase in the number and size of villi (Monari [14], Stasov [9], Bornstein [13], Novikov [7]). The stomach, duodenum and the colon also participate in compensatory processes which occur after intestinal resection. Both the motor and secretory functions of these organs undergo changes: the passage of food along the gastro-intestinal tract is slowed (Stasov [9], Lampert [4]). Mandzhgaladze [5] established on dogs that following partial resection of the intestine the solid part of the digestive juice secreted by the isolated part showed marked increase of activity of the main intestinal juice enzymes (enterokinase, phosphatase, saccharase). After resection of the lower part of the small intestine the isolated part of the intestine secretes juice in which the percentage of solid constitutents is higher than prior to resection.

Depsite the large number of papers devoted to "functional and hypertrophic compensation" (B. D. Stasov) which occurs after resection of part of the intestine, none of the investigators has carried out detailed analysis of the course of this process, restorative in character, or undertaken to clarify the mechanisms underlying it; nor has the functioning of the intestine during this period been studied.

In approaching the study of restorative processes occurring in the intestine after resection we started with the concept that the enzyme-secreting function of the intestine was reflected morphologically in manifestations of its physiologic regeneration [3]. The destructive phase of physiologic regeneration is reflected in exfoliation and sloughing of epithelial cells and sheets into the lumen of the intestine [8, 14], the sloughed epithelium, which constitutes the solid part of intestinal juice, forming the main source of the latter's enzymes [6, 10, 12]. S. L. Blyakher [2] noticed that fasting rats showed predominance of sloughing and disintegration of epithelium whereas in the case of animals which had been fed the epithelial sheet was continuous. As regards restorative processes in intestinal epithelium, it has been established that the number of mitoses in the crypts of animals which had been fed and "conditionally fed" (teasing with food) increases as compared to fasting animals (Khrushchev [11], L. Ya. Blyakher [1]).

The problem with which the present communication is concerned was to discover how the physiologic regeneration of the intestine was altered following its partial resection and what were the restorative processes occurring in it during this period.

Attention was mainly concentrated on the study of the restorative phase of physiologic regeneration. Comparison was made of the number of mitoses in the intestinal crypts of fasting animals and those which had been fed, both unoperated and subjected to resection of 1/7th of the small intestine. Such a comparison made it possible to find out whether an increase of the number of mitoses in the crypts following feeding, such as was consistently seen in non-operated animals, also occurred in the operated ones. Moreover, an attempt was made to discover whether the operated rats preserved their ability to react to natural conditioned food stimuli by means of increasing number of mitoses in the crypts.

EXPERIMENTAL METHODS

Experiments were performed on white rats weighing 200-250 g. Nine rats served as controls, the remainder (25 animals) were subjected to operation. The abdomen was opened under ether anesthesia and 8-10 cm of the intestine was resected; this constituted 1/6th-1/7th of its length. The anterior limit of the resected portion always passed 3 cm below the point of passage through the mesocolon. "End to end" anastomosis was made with the help of silk sutures. The period of observation varied in different series. When the operated animals were sacrificed a length of intestine measuring 3 cm and including the anastomosis was dissected out; a length of intestine corresponding to the site of resection in the experimental animals was removed from the control animals when they were sacrificed. The tissues were fixed in Bouin's mixture and paraffin blocks were made. Sections 7μ in thickness were stained with hematoxylin-eosin. Mitoses were counted in central longitudinal sections of 30-20 crypts; the average number of mitoses per crypt was calculated.

EXPERIMENTAL RESULTS

Six rats were sacrificed 10 days after operation; of these 3 had been fasting for 50 hours and 3 had fasted 48 hours and were then fed during 2 hours. The number of mitoses above and below the anastomosis was counted (Table 1).

TABLE 1

Number of Mitoses per One Crypt 10 Days After Operation

Number of rat	1		2		3		Average	
Nature of experiment	above the anastomosis	below the anastomosis	above the anastomosis	below the anastomosis	above the anastomosis	below the anastomosis	above the anastomosis	below the anastomosis
Fasting for 50 hours	2.8	3.0	2.9	2.9	2.7	2.7	2.8	2.9
Fasting for 48 hours + feeding for 2 hours	6.1	6.4	6.6	6.6	6.6	6.7	6.5	6.6

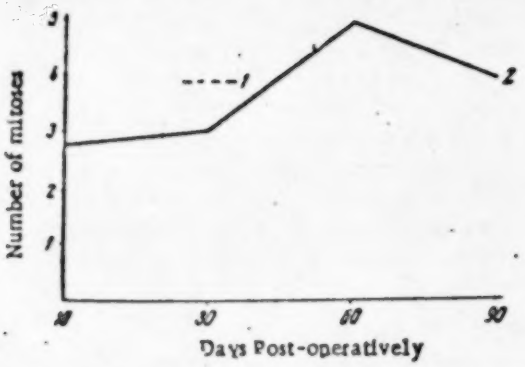
As can be seen from Table 1 the average number of mitoses per one crypt was equal to 2.8 in fasting rats and 6.5 in animals which had been fed. The number of mitoses in the regions above and below the anastomosis was approximately the same. In the case of rat No. 2 (fed) the number of mitoses in both segments was the same and equalled 6.6; in rat No. 3 (fed) there were 6.7 mitoses per crypt in the anterior portion of the intestine and 6.6 in the posterior portion; in rat No. 3 (fasting) the number of mitoses was 2.7 in both portions of the intestine.

Microscopic examination of the villi showed that marked sloughing of epithelium was taking place in the operated fasting rats, whereas in the case of the animals which had been fed almost all the villi were covered by an uninterrupted sheet of epithelium.

Analysis of data provided by the 10-day postoperative period reveals that the epithelium of the small intestine mucosa reacts to fasting and intake of food similarly to normal as early as 10 days postoperatively. Apparently holocrine secretion of enzymes occurs in the anterior and posterior portions of the intestine, which is comparable to events taking place in the unoperated animals. This can be deduced from the presence of approximately equal numbers of mitoses in the crypts of these segments as well as from the morphological features of the villi characteristic for the normal.

Consequently, 10 days postoperatively apparently no substantial changes in the processes of physiologic regeneration take place.

Thirty days following operation 18 rats were sacrificed (9 operated and 9 control). Six rats (3 operated



Changes in mitotic acitivity in the process of regeneration in the rat.
1) control; 2) experiment.

and 3 control) had fasted for 50 hours, 6 had fasted for 48 hours and were then exposed for 2 hours to the sight and smell of food (kept under netting) which they could not reach. These formed the "conditionally fed" group of animals. In the experimental rats the mitoses were counted in the segment above the anastomosis. Results of the mitotic counts are presented in Table 2.

As Table 2 indicates, the average number of mitoses per one crypt in operated fasting animals is 3.0, in animals which had been fed 5.8 and conditionally fed ones 4.5; in unoperated rats the number of mitoses is 3.9, 5.5, 5.0 respectively. The epithelium of the intestinal mucosa in the operated rats thus reacts to intake of food or being "teased" by food in exactly the same way as that which is characteristic for unoperated rats. Statistical treatment of the data showed that the differences between the values for fasting rats on the one hand and fed ones on the other were beyond the limits of chance deviation. Investigation carried out on conditionally fed animals demonstrated that the part of the small intestine remaining after resection retained the ability to react in the usual manner to the regulating influences of the control nervous system.

Two fasting and 2 fed rats were sacrificed on the 60th postoperative day (Table 3).

As in the earlier stages of the experiment, the intestinal epithelium was found to have reacted to intake of food by increasing numbers of mitoses. It is interesting that in the fasting operated rats the number of mitoses was considerably increased by comparison with their number in fasting operated animals examined 10 and 30 days after resection. This increase was statistically significant. The difference in the number of mitoses in the intestinal crypts of the operated and unoperated rats can be interpreted as an indication of the presence of hypertrophy in the portion of the intestine remaining after resection. This interpretation is confirmed by manifestations of hypertrophy, observed both macro- and microscopically. The external diameter of fixed intestine was measured. Measurements were made every 3 cm, 20 measurements on the average for every rat. The figures obtained are presented in Table 4.

TABLE 2

Number of Mitoses per one Crypt 30 Days after Operation

Nature of experiment	Operated				Unoperated			
	№ 1	№ 2	№ 3	average	№ 1	№ 2	№ 3	average
Fasting for 50 hours	2.7	3.3	3.1	3.0	3.7	4.2	3.7	3.9
Fasting for 48 hours + feeding for 2 hours	6.75	5.45	5.2	5.8	5.8	5.7	5.0	5.5
Fasting for 48 hours + conditional feeding for 2 hours	3.5	5.5	4.5	4.5	5.0	5.1	4.9	5.0

It was found that the diameter of the intestine of fasting unoperated rats was equal, on the average, to 2.7 mm and that of fasting operated rats 3.5 mm. In the case of animals which had been fed the figures were 3.5 mm for unoperated rats and 4.3 mm for operated ones. The diameter of the intestine was thus increased in the operated animals. Statistical examination of the differences in intestinal diameter showed that for fasting rats R = 5.8 and for fed rats R = 7.7. i.e., the differences were not chance. In order to discover how long

TABLE 3

Number Mitoses per one Crypt 60 Days after Operation

Nature of experiment / Number of rat	1	2	Average
Fasting for 50 hours	4.8	5.0	4.9
Fasting for 48 hours + feeding for 2 hours	5.7	6.1	5.9

intensified proliferation of epithelial cells in the small intestine crypts persisted in the operated rats 6 of the latter were examined 90 days after operation. These rats were kept fasting for 50 hours prior to being sacrificed. The number of mitoses in the crypts of these rats is given in Table 5.

Data shown in Table 5 indicate that 90 days after operation the number of mitoses in the intestinal epithelium continues to be high, although some tendency to decrease begins to appear.

Data on the number of mitoses in fasting rats sacrificed at various periods are summarized in the figure.

TABLE 4

Rat Intestine Diameter (in millimeters)

Rats	Unoperated			Operated		
	№ 1	№ 2	average	№ 1	№ 2	average
Fasting	3.0	2.5	2.7	3.5	3.6	3.6
Fed	3.4	3.3	3.4	4.5	4.2	4.4

Postoperative days are marked along the abscissa, the average number of mitoses per one crypt — along the ordinate. The curve rises until the 60th day after operation, beginning to decline from then on.

TABLE 5

Number of Mitoses per one Crypt 90 Days after Operation

Nature of experiment / No. of rat	1	2	3	4	5	6	Average
Fasting for 50 hours	4.6	4.0	3.5	4.9	2.8	3.8	4.0

On the basis of the data obtained it may be taken as established that the rat intestine, after resection of 1/7th of its length, shows definite reaction to unconditioned and conditioned food stimuli quite soon after operation, the response manifesting itself by increased number of mitoses in the crypts. The increased number of such mitoses in fasting operated rats as compared with unoperated rats (fasting) can be taken as an indication of enhanced functioning of the remaining intestine and hypertrophy of its mucosa. Following resection of part of the small intestine its remaining part thus shows the occurrence of restorative processes.

SUMMARY

1/7 part of small intestine was resected in 25 white rats in its superior portion. Intestines of rats reacted in 10-30 days after the operation by increased number of mitoses in response to unconditioned and conditioned food stimuli (as in normal condition). On the 60th day after the operation, the number of mitoses in the intestine of fasted operated rats was increased in comparison with fasted animals which were not operated,

as well as in comparison with fasted operated rats, which were sacrificed earlier. Mitosis was still pronounced in the intestine of operated rats in 90 days after intestinal resection.

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MORPHOLOGY AND PATHOMORPHOLOGY

THE ABSOLUTE NUMBER OF CELLS IN BONE MARROW AND MYELOGRAMS OF NORMAL RABBITS

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In order to evaluate correctly the functional state of bone marrow it is necessary, in addition to appraisal of a myelogram, to know the absolute number of nucleated cells in unit volume or weight of bone marrow.

The absolute number of nucleated cells is usually determined by counts on bone marrow obtained by puncture. However, when small laboratory animals are used for experiments it is not always possible to make a puncture. In those experiments in which animals are sacrificed there is opportunity to take any part of bone marrow for analysis. The present work is concerned with determination of the absolute number of cells in bone narrow of experimental animals.

EXPERIMENTAL METHODS

Experiments were carried out on rabbits aged 4-6 months and weighing 2-2.4 kg. When the animals were sacrificed, bone marrow was withdrawn from the long bones; it was placed on a watch-glass and crushed to a semi-fluid consistency, after which samples of 200 mg were taken. From each tissue sample diluted with 5% acetic acid, a suspension of cells was prepared, using the following methods: 1) titration of the tissue with a pestle in a mortar with gradual addition of the diluting fluid; 2) treatment of the tissue sample mixed with the diluent in a Waring Blender apparatus fitted with a special blunt blade; 3) preparation of suspension in a glass homogenizer; 4) taking up of bone marrow into mixing vessel and shaking with the diluent.

The cells were counted in a Goryaev chamber after a homogeneous suspension was obtained. Multiple examinations of bone marrow from 6 normal rabbits were carried out, using the methods cited for the preparation of the suspension.

The results obtained showed that the highest absolute number of cells and closest values (in parallel examinations) were obtained when the suspension was prepared in a glass homogenizer and in the Waring Blender type of apparatus.

The suspension, when prepared by these methods, is more homogeneous, the cells are evenly distributed, the time taken to prepare the suspension is very much shorter (2 minutes) than that required for its preparation by hand, when the resultant suspension is less homogeneous and contains fewer cells. When the bone marrow is taken up in a mixing vessel the figures are often close to those obtained with preparation in a homogenizer, but in some cases this method tends to give results which are too low.

Using suspensions prepared in a homogenizer and the Waring Blender type of apparatus, bone marrow cell counts (femur) were made on 22 normal rabbits. The number of cells equals, on the average, 596,000 per 1 mg tissue from 393,000 to 900,000). Since it is known that bone marrow composition varies in different bones the cell content of the following bones was determined as well on 6 rabbits: ribs, femur, humerus and tibia (Table 1); the suspension was prepared in glass homogenizer.

EXPERIMENTAL RESULTS

Table 1 shows that the highest cell content is in the rib and the lowest in the tibia. Evidently this is connected with the smaller content of fat and higher content of dry non-fatty residue in rib marrow [1].

TABLE 1

No. of rabbit	Rib	Femur	Humerus	Tibia
1	983.2	787.6	835.6	796.2
2	1 031.2	576.9	641.2	629.4
3	636.9	353.7	359.4	260.0
4	656.2	388.4	335.0	269.4
5	839.4	608.1	555.0	356.2
6	945.6	521.9	573.9	487.5
Average	848.8	539.4	550.0	466.4

In order to evaluate correctly bone marrow changes in experimental animals it is essential to know the limits of normal as regards myelograms and absolute content of various types of cells in the bone marrow, but data available in the literature are inadequate in this respect. Normal myelograms for rabbits are described by G. G. Karavanov [3]; other authors [4, 5, 6] only give normal values for peripheral blood in rabbits.

To determine the norm for myelograms, bone marrow from the femur was examined using 26 normal rabbits aged 4-6 months and weighing 2-2.4 kg (the animals were sacrificed by means of air emboli). Bone marrow smears were stained by the Pappenheim method; 1000 cells were counted on each preparation.

Data on percentage and absolute content of cells in the bone marrow of normal rabbits are presented in Table 2.

It can be seen that the main bulk of bone marrow cells is made up of cells belonging to the erythroid and granulocytic series; these are present in approximately equal proportions: total erythroblasts – 41.9%, total granulocytes – 42.4%. Neutrophils make up the greater part of the granulocytes (39.6%); the remainder (2.8%) is made up of myeloblasts (0.7%), basophils (0.7%) and eosinophils (1.4%).

According to our data cells of each succeeding generation are present in larger numbers than cells of the previous generation (with the exception of segmented neutrophils); this corresponds to the "rule of harmonic development and distribution of bone marrow cells" [2].

Among the erythroid cells are many hemoglobin-containing forms – polychromatophil and oxyphil erythroblasts. Young forms of erythroblasts constitute a total of 6.3%, the content of more mature ones being higher than that of less mature cells.

When changes in the percentage content of various generations of erythroblasts and neutrophils are to be evaluated, figures showing the percentage ratio of all the cells of a given series, the so-called fractional erythrograms and granulocytograms, are the most significant (Tables 3 and 4).

In 16 of 26 normal rabbits the absolute number of cells in 1 mg bone marrow was counted. Using myelogram data for these animals the absolute content of all types of generations of cells were calculated (Table 2). As can be seen from Table 2 the absolute number of all types of cells is given to considerable fluctuations. It appears to be impossible to determine the cause of these fluctuations on our relatively limited material or to demonstrate the relation between the absolute content of cells and their percentage ratios in the myelograms.

TABLE 2

Cellular Composition of Bone Marrow in Normal Rabbits

Name of cells		Percentage (26 rabbits examined)			Absolute numbers in thousands (16 rabbits examined)		
		minimal	maximal	average	minimal	maximal	average
	Reticular cells	0.2	1.7	1.0	1.8	12.6	6.1
	Hemohistoblasts	0.1	0.8	0.2			
Erythroblast generations	Prerythroblasts	0.2	0.8	0.2	0.8	4.9	1.3
	Basophil macroblasts	0.2	2.0	0.6	1.6	18.0	3.7
	Basophil normoblasts	0.4	10.8	5.5	10.4	58.6	33.1
	Polychromatophil erythroblasts	10.9	26.6	18.9	49.1	218.1	114.4
	Oxyphil erythroblasts	6.6	24.3	16.7	57.8	154.2	100.7
	Total erythroblasts	33.6	54.0	41.9	137.6	378.5	253.2
	Megakaryocytes	0.1	0.3	0.1	0.5	1.8	0.8
	Basophils	0.1	2.4	0.7	0.5	21.6	3.1
	Eosinophils	0.2	2.4	1.4	2.4	15.6	8.1
	Myeloblasts	0.2	1.6	0.7	1.8	8.6	4.4
Neutrophil generations	Premyelocytes	0.1	1.6	0.6	1.6	16.0	3.9
	Myelocytes	1.1	6.1	3.1	6.7	46.8	16.9
	Young	2.8	10.0	7.4	25.2	79.6	44.8
	Rod-nucleated	10.8	33.6	23.2	78.0	254.9	145.2
	Segmented	2.0	9.0	5.3	9.5	81.0	33.0
	Total neutrophils	23.0	51.6	39.6	143.9	408.9	240.8
	Total granulocytes	25.0	52.8	42.4	156.3	428.3	256.4
	Lymphocytes	4.1	21.3	12.6	22.1	149.4	68.3
	Monocytes	0.4	3.6	1.6	2.9	32.4	9.0
	Plasma cells	0.1	1.2	0.2	0.6	5.9	1.8

TABLE 3

Fractional Erythrogram for Normal Rabbits

Name of cells	Erythroblast content in %		
	minimal	maximal	average
Young erythroblasts (not containing haemoglobin)	2.6	32.7	14.9
Polychromatophil erythroblasts	27.9	64.9	44.8
Oxyphil erythroblasts	16.6	62.3	40.3

TABLE 4

Fractional Granulocytogram for Normal Rabbits

Name of cells	E Erythroblast content in %		
	minimal	maximal	average
Young neutrophils (pre-myelocytes, myelocytes, young forms)	11.0	40.7	28.2
Rod-nucleated neutrophils	41.8	70.3	58.3
Segment-nucleated neutrophils	3.9	26.2	13.5

SUMMARY

Absolute number of cells containing nuclei was determined in the bone marrow of rabbits and the ratio of various forms was calculated on myelograms. The content of cells in the bone marrow of various bones was different. The cells of erythroid and granulocytic series were in the same proportion (erythroblasts 41.9%, granulocytes 42.4%). The content of various generations of erythrocytes and neutrophils was in coordination with the "rule of harmonic development and distribution of cells, according to degree of their differentiation."

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PERIPHERAL BLOOD AND BONE MARROW INDICES IN HEALTHY DOGS

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Changes in the morphologic composition of peripheral blood and of bone marrow are very important in evaluation of the state of the organism and in various diseases as well as in various experimental investigations.

This necessitates definite knowledge of the morphologic composition of peripheral blood and hemopoietic organs in healthy animals, in particular in dogs. However, data available in the literature are inadequate. The majority of authors base their conclusions on relatively scant and heterogeneous material, so that statistical treatment shows [7] some of the differences found to be nonsignificant.

The average value given by most investigators for the number of erythrocytes is 6-7 million per 1 mm^3 of blood. The limits of fluctuation, however, allowed by some authors are very wide. A. F. Aleksandrov [1], for instance, considers the range of normal erythrocyte content in dogs to be from 3.96 to 8.4 million, while V. Bauman [2] extends it to 2.5-10 million. The same can be said concerning the color index the upper limit of which is taken to be 1.24-1.4 [5].

Reticulocytes have only been estimated by two investigators – E.C. Albritton [9] and G. A. Ryazhkin [7] whose data are widely divergent: 0-27 and 0-2.9 respectively.

The total number of thrombocytes has been determined by many authors. Average values reported have been within the range of 300,000-500,000 cells per 1 mm^3 blood with a wide scatter of fluctuations from 188,000 to 960,000 [9]. Ryazhkin [7] reports lower values for the number of thrombocytes (average – 150,000) with fluctuations from 48,000 to 250,000.

The average number of leucocytes given by many authors varies within the limits of 9,000-10,000 per 1 mm^3 of blood with fluctuations from 6,000 to 12,000 or 15,000. At the same time, A. F. Aleksandrov, E. C. Albritton and G. A. Ryazhkin take higher values as normal (12,000-13,000) with fluctuations from 7,800 to 16,800, 18,000 or 20,000.

As regards the percentage composition of the leucocyte formula, the data of most authors are consistent (60-70% cells of the neutrophil series and 20-25% lymphocytes). G. A. Ryazhkin's data form an exception: he takes 12% lymphocytes (from 3 to 18%) as normal.

The average percentage of eosinophils equals 3-6 with fluctuations from 0-3 to 6-17 while that of basophils equals 0.5-1 with fluctuations from 0 to 1.2.

The average quantity of monocytes is within the limits of 3-7% with fluctuations from 1-4 to 4-10%. Aleksandrov's data are an exception since he takes marked monocytosis (22.5%) as the upper limit of normal. Plasma cells have been determined by only three investigators – Aleksandrov, A. I. Ermolenko [3] and G. A. Ryazhkin who found them to amount to 0.4-2.25%.

The erythrocyte sedimentation rate according to A. V. Kudryavtsev [5] is within the range of 2-3.5 mm/hour and according to G. A. Ryazhkin – 6-15 mm/hour.

Information concerning the percentage content of cells in bone marrow is available in the work of several authors [1, 2, 8 and 9]. The data, however, are divergent, not related to peripheral blood findings and differ as regards cell nomenclature.

According to Stesni and Higgins, van Loon, Mulligan, Reckers, Caulter [9] and I. I. Tarasov [8] the number of erythroblast cells in the normal constitutes 40-59% and of myeloid cells – 34.5-53.4%.

According to Aleksandrov's statistics erythroblast cells make up 14-32% and myeloid cells 34.3-56.2%. Ryazhkin's figures are 30% for erythroblast and 52% for myeloid cells.

It follows from the above that fluctuations in the myeloid cell content are smaller than those in the erythroblast cell content.

Subdivision of cells according to degrees of maturation has not been done by all authors.

E.C. Albritton, analyzing the figures reported by four investigators, calculates the content of immature forms of the red and white series in percentage of the total number of bone marrow cells (100). This shows that immature erythroblasts make up 5.4% (1.8-8.4%) while immature regenerative cells of the myeloid series make up 7.4% (4-14.1%).

In view of the divergence and paucity of literature data it was decided to present the results of our investigations carried out on 200 dogs.

EXPERIMENTAL RESULTS

As can be seen from Table 1, the number of erythrocytes in the dogs examined in 6.6 million, the color index is, on the average, 0.57 and the amount of reticulocytes constitutes 8% . The absolute number of thrombocytes equals, on the average, 304,000.

1 mm^3 of blood contains 10,500 leucocytes with fluctuations from 6,000 to 14,000.

Analysis of the leucocyte formula reveals the neutrophil leucocytes make up, on the average, 64.5% (40-86%), lymphocytes – 19.5% (12-34%), monocytes – 6% (0-13%), eosinophils – 7% (0-20%) and plasma cells 0.67% (0-2%). Single basophils were only seen in a few dogs. The average ESR value was 3.6 mm/hour with fluctuations from 1 to 12 mm/hour.

TABLE 1

Normal Values for Peripheral Blood in Dogs

Index	Average values	Maximal values	Minimal values	Average quadratic deviation	Average arithmetic mean error
Erythrocytes (in millions)	6.6	8.4	5.2	±0.74	±0.05
Hemoglobin (in units)	75	94	60	±8.56	±0.6
Color index	0.57	0.76	0.50	±0.06	±0.04
Reticulocytes (in %)	8	18	1	±3.8	±0.26
Thrombocytes (in %)	48	80	25	±10.83	±0.69
Thrombocytes (in thousands)	304	500	160	±71.2	±5.03
Leucocytes (in thousands)	10.5	14.0	6.0	±1.86	±0.13
Rod-nucleated neutrophils (in %)	4	10	0	±2.28	±0.14
Segment-nucleated neutrophils (in %)	60.5	76	40	±7.52	±0.53
Neutrophils (absolute number)	6.9	10.0	3.6	±1.41	±0.09
Lymphocytes (in %)	19.5	34	12	±5.28	±0.38
Lymphocytes (absolute number)	2	3.8	0.8	±0.697	±0.04
Eosinophils (in %)	7	20	0	±4.65	±0.25
Eosinophils (absolute number)	0.7	3.0	0.2	±0.54	±0.03
Monocytes (in %)	6	13	0	±3.31	±0.23
Monocytes (absolute number)	0.6	1.6	0.2	±0.30	±0.02
Plasma cells (in %)	0.67	2	0	±0.66	±0.048
ESR (mm/hour)	3.6	12	1	±2.27	±0.227

The figures obtained by us thus approach in most cases those cited in the literature, but in some cases (total number of reticulocytes and percentage content of eosinophils) our values are higher.

Passing on to an analysis of the percentage composition of bone marrow cells (Table 2), the average values for erythroblast cells, according to our data, are 27.9%, for myeloblast cells – 57.43% and for all other cells 15.67%. Subdivision of the red and white series cells according to degree of maturation, expressing the values as percentage of total bone marrow cells (100) shows that immature regenerative cells of the erythroblast series constitute 2.3% (0.2-8.8%) while immature regenerative cells of the myeloid series constitute 5.4%. (1.2-13.4%).

TABLE 2

Normal Bone Marrow in Dogs

Index	Average values	Maximal value	Minimal value	Average quadratic deviation	Average arithmetic mean error
Hemohistoblasts	0.16	1.2	0	±0.3	±0.02
Reticulo-endothelial cells	1.0	4.0	0	±0.468	±0.03
Pre-erythroblasts	0.9	3.2	0	±0.566	±0.04
Basophil macroblasts	1.4	5.6	0.2	±1.4	±0.1
Basophil normoblasts	7.8	27	1	±4.99	±0.35
Polychromatophil normoblasts	17	34	6	±6.24	±0.44
Oxyphil normoblasts	0.8	4.8	0	±0.09	±0.07
Total number of erythroblast cells	27.9	44	16		
Mitoses in red series	1.0	2.4	0	±0.59	±0.04
Myeloblasts	0.9	3.0	0	±0.64	±0.04
Premyelocytes	1.9	4.4	0.2	±1.04	±0.07
Myelocytes	2.6	6	1	±0.95	±0.06
Young	6	18	2	±3.18	±0.22
Rod-nucleated neutrophils	27	48	6	±7.55	±0.53
Segment-nucleated neutrophils	15	42	6	±7.81	±0.55
Eosinophils	4.0	10.0	1	±1.87	±0.13
Basophils*		0.8	0		
Total number of myeloid cells	57.43	74	40		
Lymphocytes	4	12	0	±2.66	±0.19
Monocytes	2.5	11	0	±2.19	±0.15
Megakaryocytes*		1.2	0		
Megakaryoblasts*		0.8	0		
Mitoses in white series*		1.6	0		
Ferrata cells*		1.2	0		

* Average values were not calculated because of large numbers of zero values.

Analysis of fractional composition showed that young erythroblasts (pre-erythroblasts and basophil macroblasts) constituted 8% of the total number of erythroblast cells and young elements of the myeloid series (myeloblasts, premyelocytes and myelocytes) constituted 9% of the total cells in the white series.

Thus, our findings indicate that in the bone marrow of healthy dogs (mongrels) myeloid elements predominate over erythroblast elements, and mature forms over immature forms.

At the same time qualitative changes, described in detail by A. P. Egorov [4], were also encountered in animals. We found in smears of peripheral blood: hypersegmentosis 0.2% (0-3.5%), fragmentation 1.4% (0-8%), giant forms 0.14% (0-3%), chromatolysis 0.73% (0-4%) and granulocyte cytolysis 1.24% (0-6%), and agranulocytes 1.43% (0-8%). Bone marrow smears showed: pyknosis 2.52% (0-10.6%), chromatolysis 1.07% (0-7.8%), rhexis 0.13% (0-1%), giant forms 0.17% (0-5.5%), hypersegmented forms 0.13% (0-1.2%), fragmentation 0.36% (0-3.2%), vacuolization of cells 0.17% (0-1.6%), cytophagia 0.03% (0-0.7%), pigment 0.12% (0-1.2%), cytolysis 13.4% (2-35%).

SUMMARY

Determinations of various indices of peripheral blood and bone marrow of 200 healthy dogs were analyzed statistically and are presented in this paper. The average values for erythrocytes (6.6 million), leukocytes (10,500), thrombocytes (304,000), reticulocytes (8%) and erythrocyte sedimentation rate (3.6 mm per hour) are very close to those mentioned in literature. Numerical values, reflecting the contents of erythroblastic (27.9%) and myeloid (57.43) elements of the bone marrow differ to a certain extent from the corresponding data of I. I. Tarasov and A. F. Alexandrov. The numerical indicators of qualitative changes of the cells of peripheral blood and bone marrow are presented in this work.

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HISTOCHEMICAL CHARACTERISTICS OF MORPHOLOGICAL REACTIONS ENCOUNTERED IN BRUCELLOSIS

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Morphological studies of the pathogenesis and immunogenesis of Brucella infection have shown that resistance to infection is based on a phagocytary defense mechanism [4, 5].

Cytomorphological studies of the reaction to live vaccines have shown that the development of active immunity to brucellosis is associated with a strong macrophage reaction and with hyperplasia of elements of the reticulo-endothelial system; Such a reaction should necessarily involve biochemical changes in the cells of the reticulo-endothelial system.

We have concentrated our attention on the study of nucleic acids, in view of the importance of the role played by them in the vital activities of cells.

Numerous histochemical investigations have established the importance of the part played by nucleic acids in the synthesis of proteins and the growth and division of cells.

The work of Ehrich, Drabkin, and Forman [12] has revealed the relation between production of antibodies and changes in the nucleic acid content of lymph nodes located near the site of injection of vaccine. Maximum content of ribonucleic acids in the cells coincided with maximum production of antibodies in the lymph glands.

T. Harris and S. Harris [13, 14] obtained similar results. They also report the interesting observation that rise in cell ribonucleic acids does not take place following introduction of nonantigenic substances (lanolin , oil emulsions).

Papers on the disribution of nucleic acids in the presence of an active tuberculous process have appeared of recent years in Soviet journals.

A. Ya. Fridenshtein [10] and M. M. Averbakh [1] have shown that during the early stages of development of the inflammatory process in tuberculous infection hyperplastic reticular and other cells become enriched in ribonucleic acid, but with further development of the tuberculous lesions the ribonucleic acid content of the cytoplasm of the cells diminishes.

M. P. Pokrovskaya [8] has demonstrated the existence of a mutual relationship between phagocytary processes and cell ribonucleic acid content.

On the assumption that the content of nucleic acids in cells is correlated with functional state of organs and tissues, we investigated the amount and topographic distribution of ribo- and deoxyribo-nucleic acids in cells of the lymph glands and spleen, in infected and immunized animals.

We applied the Brachet reaction to the histochemical study of ribonucleic acids (RNA), and the Feulgen reaction to the study of deoxyribonucleic acids (DNA).

The present research includes data on three groups of guinea pigs: vaccinated, revaccinated, and infected. In all 100 animals were studied, at various times after vaccination or infection (1 – 2 – 5 – 10 and 30 days, and 3 and 6 months.)

The guinea pigs were vaccinated with the strain Br. abortus$_{19}$, at a dosage level of 10^9 cells, subcutaneously.

Subcutaneous revaccination was effected with the same strain, using a dose of 500 million cells, 3 and 8 months after primary vaccination. The animals were infected with the virulent strain Br. melitensis$_{548}$, introducing a dose of 10 cells.

The following results emerged from a study of the distribution of RNA and DNA in cells of the reticulo-endothelial system, during development of the vaccinial process.

During the first few days after vaccination with a dose of 1 billion microbial cells we observed a generalized lymphoid hyperplasia, with sporadic clear cells, in the lymphatic glands and spleen. This morphological reaction was not associated with change in the RNA and DNA contents of the cells. The reticular cells contained very little RNA. As in normal animals, RNA was found chiefly in the lymphoblasts and lymphocytes.

The RNA content of the cells changed with increasing intensity of the reaction in the lymph nodes and spleen, characterized by increase in the size of centers of multiplication of follicles of lymph glands, and by generalized large cell hyperplasia. Large numbers of reticular cells, with a high RNA content of the cytoplasm, and with well-defined nucleoli, appear at these centers.

The number of lymphoblasts containing RNA in the cytoplasm increases. On the 15 – 30th day after vaccination isolated small nodes of reticular and epithelioid cells make their appearance, against a background of large-cell hyperplasia. The cytoplasm of reticular cells becomes impoverished in RNA during their transition into epithelioid cells. Sporadic reticular cells, which lose their connection with the reticular stroma, change into polyblasts, and have RNA-rich cytoplasm. The phagocytary function of these cells (macrophages) is, however, associated with fall in cytoplasmic RNA content.

At longer times after vaccination (6 months) the reticular cells of the lymph nodes and spleen have little RNA in the cytoplasm, but a high content in the nucleoli.

The plasma cells are of particular interest. By the 10 – 15th day after vaccination large numbers of plasma cells are encountered in the lymph glands and spleen. In the earlier post-vaccinial stages juvenile forms of plasma cells are seen, with a scant cytoplasm of a high RNA content, but as the process of generalized cell hyperplasia develops the total volume of protoplasm also increases, and together with it its RNA content.

The plasmocellular reaction in the lymph nodes is now related to synthesis of antibodies. A. Fagreus [11], in his painstaking researches, has demonstrated the relationship between increasing basophilicity of plasmocytes and their antibody content. The presence of plasma cells with a strongly basophil protoplasm, after immunization with live antibrucellosis vaccine, merits special attention, since we have encountered this type of reaction only during development of the vaccinial process.

A rapid response reaction is seen in the reticulo-endothelium of lymph glands and the spleen after vaccination of guinea pigs, 3 and 8 months after primary vaccination.

A pronounced large cell hyperplasia, with numerous mitoses, and formation of macrophages, is evident in the lymph glands within 2 hours. Large numbers of polyblasts and plasma cells make their appearance. Similar effects are seen in the spleen.

This morphological reaction is associated with accumulation of RNA in these cells. Its content rises considerably in the cytoplasm of the reticular cells (Fig. 1). The nucleolus present in the nuclei of these cells increases in size, and contains very much RNA. Cells containing two or three distinct nucleoli are very frequent.

Such an enrichment of the reticular cells with RNA points to their exceptional activity. Reticular cells displaying phagocytary activity lose part of their RNA in the cytoplasm, but they still have a higher RNA content than do the macrophages encountered during primary immunization. Other cells present in the lumph nodes,

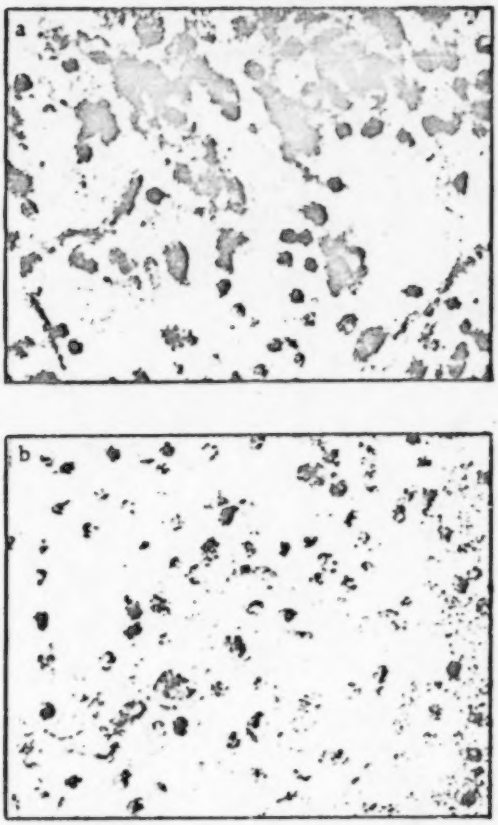

Fig. 1. Lymph gland of a revaccinated guinea pig. a) reticular cells, with a high RNA content; b) the same section, treated with ribonuclease. Magnification × 660.

viz., lymphoblasts, polyblasts, and plasma cells, are the richest in RNA (Fig. 2). The distribution of RNA within the lymphoblasts is very often irregular. During the stage of intensive hyperplasia of the follicles at proliferation centers we encountered numerous macrophages containing large spheroidal inclusions, which stained deeply with pyronin, in contrast to the faintly staining cytoplasm. These inclusions were absent after treating the sections with ribonuclease, showing that they consisted of RNA. It is not yet clear whether this RNA originated from the macrophage itself, or whether it represents phagocytized cell debris. We were unable to find any significant shifts in the DNA contents of the cells. It should, however, be noted that the DNA content of the lymphoid cells increased as the vaccination process developed. The DNA content of the nuclei of the reticular cells also increased somewhat, while the epithelioid cells contain practically no DNA, the little there is being distributed in the form of very small granules at the peripheries of the nuclei. The DNA content of the nuclei rises during the process of mitotic division of the cells.

Simultaneously with the vaccinated animals, we observed a group of guinea pigs infected with a virulent culture of the strain Br. melitensis$_{548}$ (inoculation dose 10 microbial cells). We studied the distribution of nucleic acids in elements of the reticulo-endothelial system during development of the infection.

During the first 5 – 10 days after inoculation we observed a generalized lymphoid and large cell hyperplasia of cells of the reticuloendothelium of lymph glands and of the spleen, with approximately the same RNA contents as were encountered in primary vaccination. The RNA content of the cells then fell, as the pathological process developed, with formation of large epithelioid proliferates, occupying 90-95% of the lymph nodes, and forming specific granulomata. The epilthelioid cells all had a low RNA content, and RNA was practically absent from epithelioid and giant cells of the specific granulomata, and diminished in the lymphoblasts.

The nuclei of most of the epithelioid cells either had no nucleolus, or had only a faintly discernible one.

The data on the distribution of RNA in elements of the reticuloendothelium of infected animals indicate that the functional activity of these cells is considerably lowered.

Our cytological study of formation of immunity to brucellosis showed that high and stable immunity can be achieved with a dose of 10^9 living microbial cells. Formation of immunity is associated with an intense macrophage reaction, and with vaccinial hyperplasia of elements of the reticulo-endothelial system, which is most pronounced on the 10 – 15th day after vaccination.

By that time the guinea pigs are immune to infection with virulent strains of Brucella.

The hyperplasia observed in the reticulo-endothelium during the development of the vaccinial process taking place after inoculation with 10^9 microbial cells is associated with accumulation of ribonucleic acid in the cytoplasm and nucleoli of reticular cells.

The RNA content of all the cells of lymph nodes and of the spleen is at a maximum on the 15 – 30th day after primary vaccination, and on the second day after revaccination.

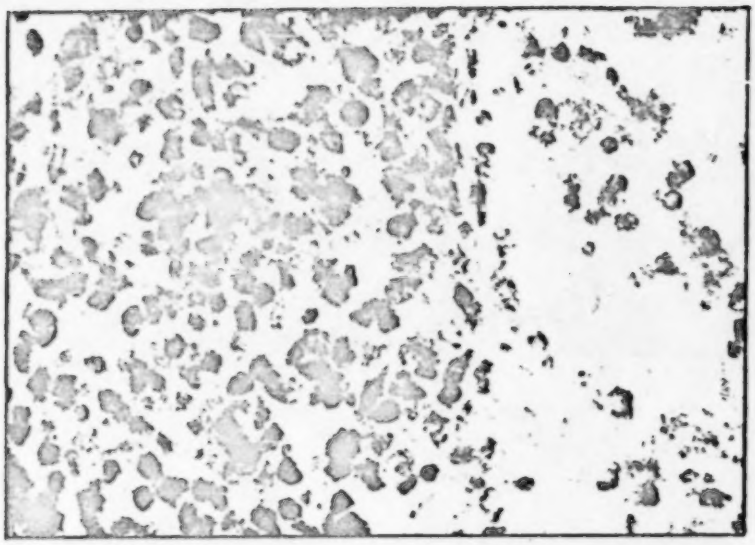

Fig. 2. Lymph gland of a revaccinated guinea pig. Plasma cells, rich in RNA. Magnification × 660.

Inoculation of guinea pigs with a virulent strain of Br. melitensis leads to an infection causing pronounced pathological changes in the organs, associated with a fall in the cell RNA content.

Thus the hyperplasia of reticulo-endothelial elements, observed in response to live vaccine is associated with increase in the RNA content of reticular cells, pointing to the enhanced functional activity of these cells in the synthesis of proteins.

The work of B. V. Kedrovsky [6, 7], A. N. Belozersky [2], G. I. Roskin [9], G. Casperson, J. Brachet [3], A. Fagreus [11], and others has provided convincing evidence of the direct connection of ribonucleic-acid with the synthesis of proteins and specific antibodies.

We are justified, on the basis of the work of these authors, in drawing the conclusion that vaccinial hyperplasia of reticulo-endothelial elements, in response to live vaccine, involves intensification of cell metabolism, leading to reconstruction of intracellular enzyme systems and to formation of specific globulins.

SUMMARY

Production of immunity in brucellosis as a result of introduction of live vaccine is associated with severe macrophagic reaction, as well as with vaccinial hyperplasia of reticulo-endothelial elements.

Intensive accumulation of ribonucleic acid in the cytoplasm and in the nuclei of reticular cells takes place during the process of vaccinial hyperplasia of reticulo-endothelium. Phagocytic function of macrophages is connected with reduction of ribonucleic acid in their cytoplasm. The largest quantity of ribonucleic acid in all the cells of the lymph nodes and the spleen is noted within 15 to 30 days following initial vaccination. The same picture is revealed on the second day after revaccination which is carried out in 3 months.

Brucellosis induced in guinea pigs by virulent culture of Br. melitensis causes pronounced pathological changes in the organs and is associated with reduction of content of ribonucleic acid in the cells.

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VITAMIN C CONTENT OF LABORATORY ANIMALS, AS SHOWN BY HISTOCHEMICAL METHODS

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Evidence has been published that certain drugs affect the ascorbic acid content of animal organs. It has been shown that aspirin, sulfanilamide, and potassium iodide cause a fall in the vitamin C content of human blood, and that administration of sulfanilamide to guinea pigs and albino rats leads to accumulation of ascorbic acid in the liver [4, 5]. Medicinal doses of quinine hydrochloride, sodium salicylate, and calcium chloride cause depletion of body ascorbic acid [2], while barbiturate derivatives stimulate biosynthesis of vitamin C [1].

H. Steege and R. Pirtkin [10], using a histochemical method, have shown that the ascorbic acid content of the adrenal glands rises after administration of thiosemicarbazones.

On the strength of these published data, we decided to apply the histochemical method to the detection of ascorbic acid in animal organs, and to use this as a test in the comparative study of the action of drugs.

EXPERIMENTAL METHODS

We applied the method of Giroud and Leblond [7] to the detection of ascorbic acid in tissue sections. Small fragments of tissue are immersed in a 10% silver nitrate solution, made acid with 1% acetic acid, and reduction of silver nitrate takes place within 15-30 minutes. The tissue is then withdrawn from the solution, washed with distilled water, and fixed in 3% hyposulfite solution for 15-30 minutes, after which it is again washed, and embedded in paraffin in the usual way. Black granules of silver are seen in the tissue sections at the locations of ascorbic acid in the fresh tissue. G. I. Roskin [3] and Burne [6] regard the reaction as being specific for ascorbic acid, under the given conditions. All manipulations involving silver salts were performed in a dark room, using a red light.

We know of only very few Soviet and foreign papers on the histochemical study of the distribution of vitamin C in animal and human organs [3, 8, 9], and they do not give a complete picture of the morphological distribution of ascorbic acid in organs.

The present paper gives an account of an investigation of the distribution of ascorbic acid within the cells of various organs of laboratory animals.

Results of Histochemical Studies

We examined the organs of 30 guinea pigs, 20 white rats, and 14 cats.

We found high ascorbic acid contents in the intestine, liver, adrenals, hypophysis, and gonads, and less in the nervous system, myocardium, and skin; it was absent from skeletal muscle, connective tissue, and thyroid gland.

In the alimentary tract, the highest ascorbic acid contents were found in the mucosa of the duodenum and of the higher levels of the jejunum; the mucosa of the stomach and the colon contained little ascorbic acid.

The distribution of ascorbic acid in the mucosa of the small intestine varied for different animals. In guinea pigs maintained on a fully adequate diet, vitamin C is indicated by small or large deposits of silver: fine, dust-like granules of silver are seen in the protoplasm and cuticles of the mucosal epithelium, in smooth muscle fibers, and in intramural ganglion cells; both fine and coarse granules are seen in mesenchymal cells of the reticular stroma of the villi and lymphatic follicles, and at the poles of the nuclei of endothelial cells of the blood and lymph vessels (Fig. 1, a).

Fig. 1. Deposition of silver granules at the sites of concentration of ascorbic acid in the epithelium of an intestinal gland of a normal guinea pig (a), and in the Golgi apparatus of an albino rat (b).
Method of Giroud and Leblond. For (a) ocular × 10, obj. × 10; for (b) ocular × 10, obj. × 90.

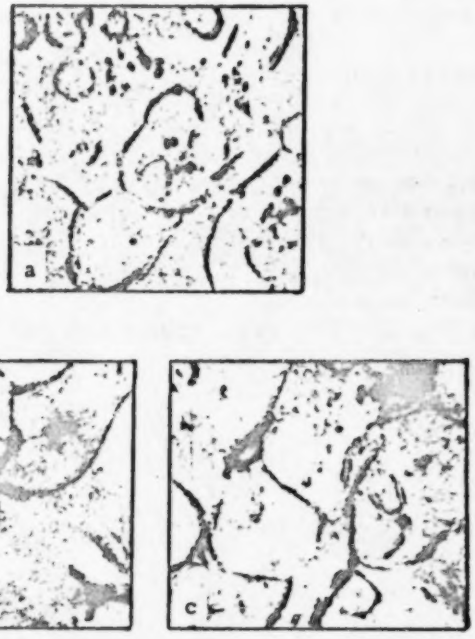

Fig. 2. Various structures of silver deposits at sites of concentration of vitamin C: a) in guinea pig liver cells; b) in cat liver cells; c) in Kupfer cells.
Method of Giroud and Leblond. Magnification: for a) and c), ocular 10 ×, obj. 40 ×; for b) oc. 10 ×, obj. 90 A.

A similar, but denser, distribution of ascorbic acid in cells of the intestinal wall of albino rats was found. In addition, we observed dense aggregations of granules in the Golgi apparatus of epithelial cells of the intestinal glands; these formations resemble those described by Tonutti (Fig. 1, b).

Various forms of deposits were seen in the liver. The granules are diffusely distributed in the parenchyma cells of guinea pigs, and are very small; complex structures made of granules and spherules appear in groups of cells (Fig. 2, a). Polygonal masses were seen in the cytoplasm of hepatic cells of albino rats and cats (Fig. 2, b).

Fig. 3. Silver deposits at sites of concentration of vitamin C in cells of the cerebral cortex of a normal guinea pig.
Method of Giroud and Leblond. Magnification: oc. 10 ×, obj. 90 ×.

The Kupfer cells of guinea pig liver contained isolated small granules, and of albino rats and cats large granules, singly, or in aggregates (Fig. 2, c).

Vitamin C was encountered in reticular cells of the spleen follicles and pulp, sometimes in large amounts in albino rats. It is present in the cytoplasm of epithelial cells of the convoluted tubules of the kidney, and less frequently in that of the straight tubules. It is encountered in the myocardium, chiefly in the myofibrils and in the endothelium of the blood vessels (as granular structures).

The ascorbic acid content of the skin varied according to the sampling site; high concentrations were found in the skin of the nose and of the plantar surfaces of the extremities of albino rats, and lower ones in skin taken from the trunk or from other parts of the limbs. The granules were present in the cytoplasm of the glandular epithelium, the fibroblasts, and the fat cells, and fine granular structures were seen in the ground substance.

A survey of the endocrine glands of adult animals showed that ascorbic acid could not be detected in the cells of the pancreas and the thyroid, but was present in the adenohypophysis, in the interstitial cells of the testes, and in luteal and follicular cells of the ovary, as shown by silver granules. Its distribution in the adrenal glands has been described by Giroud [8, 9] and others, but in insufficient detail. We found that ascorbic acid was present in both the cortex and the medulla of the adrenal glands of normal animals; its distribution in the cortex was not uniform. The ascorbic acid content of the cells of the glomerular zone varied widely in different segments. In the fascicular zone it was not infrequently concentrated around the capillaries, the lumina of which contained small amounts of ascorbic acid. The highest concentrations were found in the cells of the reticular zone, some of which showed a few granules only, while in others the cytoplasm was filled with granules and aggregates, to such an extent that they obscured the nucleus. Vitamin C appeared in the form of very fine to medium granules in the large cells of the adrenal medulla, and in the medullary sympathetic nerve cells. In addition, ascorbic acid could be detected in the reticular cells of the walls of the cortical capillaries, which contained more or less sparsely scattered granules.

Ascorbic acid was found in small amounts in the central nervous system of adult animals, chiefly in the cytoplasm of cortical nerve cells, in the cells of the subcortical nodes, and in the motor cells of the anterior horn of the spinal cord. Isolated small granules were seen in the endothelium and lumen of capillaries and blood vessels of the brain (Fig. 3).

We were never able to find ascorbic acid in the nuclei of cells of normal tissues.

Tonutti [11] believes that ascorbic acid is at first deposited in the form of large structures in the Golgi apparatus, from whence it is supplied to other parts of the cytoplasm as required. In his opinion, the Golgi apparatus serves as a bridge for the subsequent passage of ascorbic acid into the protoplasmic substance, which it protects from the effects of sudden rise in ascorbic acid content, due to uptake from the blood stream.

The finely granular vitamin C structures are encountered in the protoplasm and peripheral parts of the

endothelial cells of the intestinal glands, in which absorption of monosaccharides and synthesis of disaccharides takes place, in the liver cells, in muscle fibrils, and in the cytoplasm of nerve cells of the vegetative system and of the anterior horns of the spinal cord, which have a high metabolic rate.

It is conceivable that this finely granular form of vitamin C is associated with carbohydrate metabolism of the tissues.

The ascorbic acid content of cells is subject of considerable variations, depending on the functional state of the organ. We found high concentrations of ascorbic acid in the tissues and cells of embryos, including those of the pancreas, which is practically free of ascorbic acid in the adult.

Variations in the ascorbic acid contents of organs of experimental animals to which various drugs had been administered will be reported in subsequent communications.

SUMMARY

Depositions of silver were revealed at the sites of appearance of ascorbic acid in the organs and tissues of white rats, guinea pigs and cats by the histochemical method of Giroud and Leblond. It was established that the distribution of ascorbic acid and its concentration in the organs was subjected to variations depending on introduction of different drugs. The multitude of forms of deposited ascorbic acid was demonstrated in different cells. It was found in the form of dust, granules, lumps, spheres and complicated figures. An attempt was made to find some association between these forms and various types of metabolism. It was established that the distribution of ascorbic acid and its concentration in the organs was subjected to variations depending on introduction of different drugs.

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MORPHOLOGY OF CALCULOUS CHOLECYSTITIS

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In most of the researches devoted to the study of the morphology of calculous cholecystitis attention has been focussed chiefly on changes in the epithelium and subjacent tissues of the gall bladder; insufficient attention has been paid to changes in the intramural nervous apparatus, although quite detailed studies have been made of the normal structure of the intramural nervous system, and of its relations with other tissues elements of this organ [13].

There are also numerous published papers in which the outstanding importance of functional changes in the nervous system for the pathogenesis of extrahepatic bile duct disease has been indicated [15].

We could, however, find only two published papers describing pathological changes of the intramural nervous apparatus of the gall-bladder in chronic cholecystitis. The neurohistological studies described in these papers were, however, conducted without sufficient regard to the state of other tissues of this organ [13, 17]. Apart from this, the material on which these studies were based was derived from cholecystectomy operations, and could not throw much light on the dynamics of the changes in the intramural nervous apparatus under inflammatory conditions, particularly in the early stages of the inflammatory process. The elucidation of the interrelations between changes in the intramural nervous system and in other tissue elements involved in the inflammatory process, as well as of the sequence of these changes, is of practical and theoretical importance, and can be achieved only by an experimental approach. With this object, experiments were performed on 90 guinea pigs, in which a model condition of cholelithiasis had been induced, by the method of N. N. Petrov and N. A. Krotkina [11], with some minor modifications.

EXPERIMENTAL METHODS

The animals were operated under morphine-ether anesthesia, under conditions of strict asepsis. After laparotomy, the gall-bladder was withdrawn from the incision, an opening was made in the fundus, and a sterilized mass of small glass beads, held together by thin copper wire, was introduced into the lumen of the gall-bladder. The gall-bladder and anterior abdominal wall wounds were than closed. The animals were killed by exsanguination at various times after the operation: 30 minutes, 6 hours, 12 hours, 2, 5, 8, 9, 12, 15, 25, 33, 45, 60, 80, 107, 140, 172, 210, 265, 317, 381, 420, 453, 510, 578, 612, and 680 days. The gall-bladder was immediately removed, and immersed in 12% neutral formalin. After fixing for 5 days, half of the organ was impregnated with silver, by Bielschowsky's method as modified by Gross, and the other half was dehydrated and embedded in celloidin-paraffin. The histological sections were stained with hematoxylin-eosin, picrofuchsin, and mucicarmine. Some sections were stained with iron hematoxylin, according to Heidenhain,.

EXPERIMENTAL RESULTS

The nervous elements of gall-bladder did not show any morphological changes during the early stages (up to 2 days) of the acute inflammatory process.

The epithelium was desquamated, remaining intact only in the mucosal folds. The mucosa and muscle coats were edematous, and the vessels were engorged with blood, with marked marginal stasis of leucocytes. A fair number of leucocytes was seen in the edema fluid between the tissue elements. By the 5-8th postoperational day the epithelium surviving in the depths of the mucosal folds had begun to regenerate rapidly, and to cover the rugae. During this regenerative process, and in particular in its early stages, dedifferentiation of the epithelial cells was evident; they lost their cylindrical form, and became flattened. The cell boundaries were obliterated in the growth zone and adjacent parts, and the plates closing the epithelial cells were not apparent. Instead of being located at the base of the cells, as is usual for endodermal epithelium, the nuclei were situated at the most diverse levels. As the process continued, the edematous mucosal rugae became covered with regenerating epithelium, the height of which increased; the cells were at first cuboidal, and later on columnar, and mucinogen droplets appeared. Such secondary differentiation of regenerated epithelium was seen only in those areas of the mucosa which were not in direct contact with the foreign body; where such contact existed, the epithelial cells remained squamous, and mucinogen droplets were not seen in them.

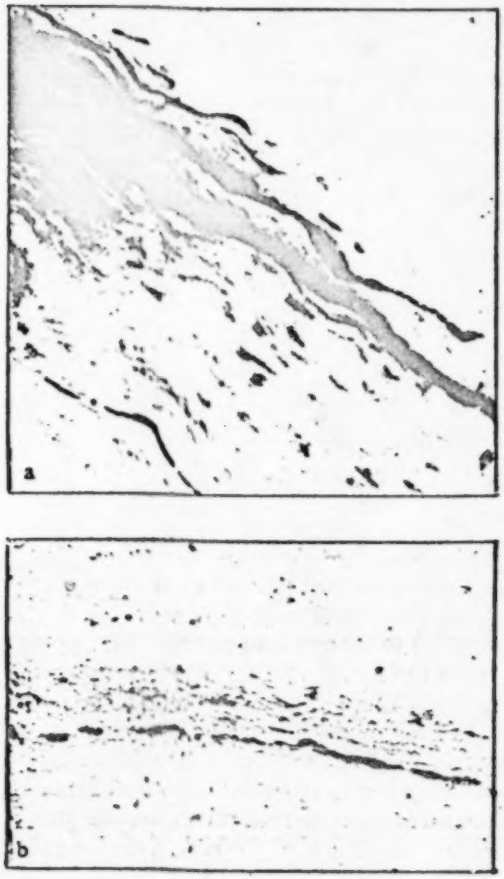

Fig. 1. Changes in nerve fibers of the muscle coat of the gall-bladder in experimental cholecystitis. a) varicose swelling and enhanced argentophilia of nerve fibers. Eight days after operation. b) disintegration of a nerve fiber, 60 lays after operation. Impregnated according to Bielschowsky-Gross. Magnification: a) oc. 8 ×, obj. 40 ×; b) oc. 8 ×, obj. 8 ×.

By this time the first morphological changes had appeared in the intramural nervous apparatus, in the form of heightened argentophilia of the nerve fibers, demyelination of the areolar nerve fibers, and thickening of the receptors (Fig. 1, a). There were no visible morphological changes in the nerve cells of the intramural ganglions.

After the 8th day we could not distinguish receptor formations in the mucosa, although they were still evident in the muscle and subserosal layers of the gall-bladder.

By the 12th day changes in the structure of the mucosa had become evident. The great majority of the epithelial cells of the rugae and of the crypts were overloaded with mucinogen. Intensified new formation of mucous glands was seen at the same time.

In the normal guinea pig gall-bladder the mucous glands are isolated, and are located in the depths of the crypts, mostly in the vicinity of the neck. They are rarely found in the body and the fundus of the bladder. In their structure, they resemble the alveolar type of gland [5].

We were able to trace the new formation of mucous glands in both the body and the fundus of the gall-bladder, not only in the depressed parts, but also higher up, on the sides of the rugae. Pre-existing glands, of primitive structure, also underwent development, to the more complex tubuloalveolar type.

As the inflammatory condtion progressed from the acute to the chronic state, we observed, apart from proliferation of connective tissue and glands, a hypertrophy of the muscle coat, as a result of which the wall of the gall-bladder had attained a thickness of 3.5 mm by the 60th day. By this time the changes in the intramural nervous apparatus were more pronounced. Receptors had disappeared not only from the mucosal, but also from the deeper layers of the walls of the gall-bladder. Irreversible changes were evident in the majority of the nerve fibers, in the form of fragmentation and

disintegration (Fig. 1b). At the same time as dystrophic changes were seen in most of the nerve fibers, we observed a weak tendency towards regeneration of unmyelinated nerve fibers in the muscular coat of the gall-bladder. These regenerated fibers are, however, unstable, and we observed varicose swelling, fragmentation, and disintegration of such fibers. This coincided with intensification of the inflammatory proliferation of the epithelium with formation of glands. These glands were formed not only in the proliferating connective tissue of the mucosa, but also within the muscular layer, down to the serous coat of the gall-bladder; glandular and cystic structures appeared, with a pronounced tendency towards differentiation into mucous glands. By the 80th day, the whole wall of the gall-bladder was penetrated by a mass of intricately ramified tubulo-alveolar glands. The ducts leading from these glands are lined with epithelial cells from the surface musoca, and are known as Luschka ducts, the intensive formation of which was seen on the 30th day after the operation. Retention cysts very often formed, owing to hindered outflow of secretion. The trapped secretion undergoes condensation, and adsorbs bile pigments and calcium salts, giving rise to formation of intramural bile pigment and calcium stones (Fig. 2).

Fig. 2. Wall of cyst in a newly formed mucous gland. The lumen contains condensed secretion, with deposits of calcium salts and bile pigments. Specimen taken 107 days after operation. Stained hematoxylineosin. Magnification: oc. 8x, obj. 8 x.

Dystrophic changes were evident by the 107th day, consequent to the continuing irreversible degenerative changes in the intramural nervous apparatus and to the hypertrophy of the muscular coat. Vacuoles of various sizes were seen in the muscle fibers, which showed homogenization of parts of the protoplasm, with granular disintegration and pyknotic nuclei. In such locations we observed proliferation of connective tissue between the degenerate muscle fibers, initially rich in cellular elements, but eventually maturing into cicatricial tissue. By the 172nd day, the whole of the wall of the gall-bladder had undergone transformation into scar tissue, with sporadic inclusions of muscle fibers and of cystic formations distended with secretion, being mucous gland ducts. It was only at this stage that we observed changes in the intramural ganglion nerve cells. Large vacuoles appeared in some of them, and masses of different sizes, staining intensively with silver, in others. Nerve cells not showing these changes were smaller than normal. We were not able, in all such cases, to demonstrate the presence of cell processes by silvering techniques. Activation of satellite cells was apparent around the most degenerate nerve cells. Unmyelinated nerve fibers entering the adventitia of arteries supplying the gall-bladder survived longer than other nervous elements.

Thus the inflammatory process brings about a total reconstruction of the wall of the gall-bladder, involving profound changes affecting its basic elements. The changes seen in the intramural nervous apparatus are of secondary origin, i.e., they appear as the inflammatory process progresses. In the early stages, when the

inflammatory reaction was of acute nature, we were unable to find any morphological changes in the nervous system of the gall-bladder. In the later stages these changes, being of secondary origin, could undoubtedly affect the development of irreversible changes in other tissues, during the reconstruction of the walls of the gall-bladder resulting from the intercurrent low-grade inflammatory process.

The resistance of nervous formations to acute inflammatory conditions has been noted by a number of authors [1, 2, 3, 8, 14]. Low-grade inflammatory processes, however, appear to involve profound changes in the nervous system [1]. It is however, recognized that inflammatory processes in denervated tissues are characterized by their extreme indolence [4, 9, 10, 17 and others). Attention has of recent years been directed to the role of the sensory neuron in the development of tissue reactions. Thus, according to T. A. Grigoryeva [4] organs deprived of their sensory connections resemble foreign bodies, of which the organism tries to rid itself by means of development of inflammatory processes within them. According to E. I. Zlotnik [7] and to E. A. Rudnik (1952), denervation of glandular organs leads to a profound reconstruction of the mucosal coats. Formation of mucous glands producing large amounts of mucous secretion takes place during this reconstruction process. Motor denervation of smooth muscle has been shown by I. P. Lukyanova [10] to lead to acute necrobiotic changes.

I. D. Khlopina [16] has found that denervation causes a disturbance in the mutual relations between the epithelium and the underlying tissues, thus interfering with acievement of regeneration, owing to which the healing of neurotrophic ulcers proceeds sluggishly, and in an irregular manner.

In our experiments, reconstruction of the gall-bladder mucosa was observed to coincide with disappearance of receptor formations and nerve fibers. The indolent course of the inflammatory process observed by us in the late stages of our experiment is evidently to a large extent due to denervation proceeding during the inflammatory process. It follows that the intense inflammatory proliferation of the epithelium is due rather to the profound dystrophic changes taking place in the underlying tissues of the gall-bladder epithelium as a result of denervation, than to injury of the epithelium by the foreign body introduced into the gall-bladder. This is because the foreign body loses its mobility as the inflammatory process passes into the chronic stage, becoming fixed in position by epithelial outgrowths. This fixation reduces its harmful action to a minimum. This is in agreement with the views of N. N. Petrov and N. A. Krotkina [11], who found pronounced epithelial proliferation even after the foreign body introduced into the gall-bladder had been ejected into the intestine.

According to G. N. Zabusov and I. F. Ivanov [6], the possibility of regeneration of the intramural nervous apparatus of the gall-bladder after its destruction in the course of an inflammatory process is inconsiderable. We, too, noted the limited extent of regeneration of nerve fibers in the walls of the gall-bladder, and the instability of the regenerated fibers. It is possible that the denervation occurring during the process of chronic inflammation of the gall-bladder removes tissue processes taking place in this organ from central nervous control, and may to some extent prepare the ground for the development of cancer.

SUMMARY

Experimentally induced cholecystitis was studied in guinea pigs at periods varying from several minutes to 680 days. It was established that the intramural nervous apparatus of the gall bladder does not show any morphological changes in acute stage of the inflammatory process. Degenerative changes develop at first in the receptor apparatus and later when the inflammatory process changes into subacute and chronic phase they appear in the nerve fibers. This process terminates by disintegration of the latter. In the late stage of this experiment the nerve cells of intramural ganglia under dystrophic changes.

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METHODS

AN APPARATUS FOR RECORDING BALLISTOCARDIOGRAM DISPLACEMENT RATES AND ACCELERATIONS

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The ballistocardiograph, being a valuable diagnostic tool, is being widely introduced into cardiovascular clinics.

There are at present two types of apparatus, in which the ballistocardiogram is recorded indirectly and directly, respectively. Of the indirect the most common are Starr's high frequency table [4] and Nickerson's low frequency table, critically damped [2]. These can record only two parameters of the motion, velocity and displacement, the third – acceleration – not being recorded.

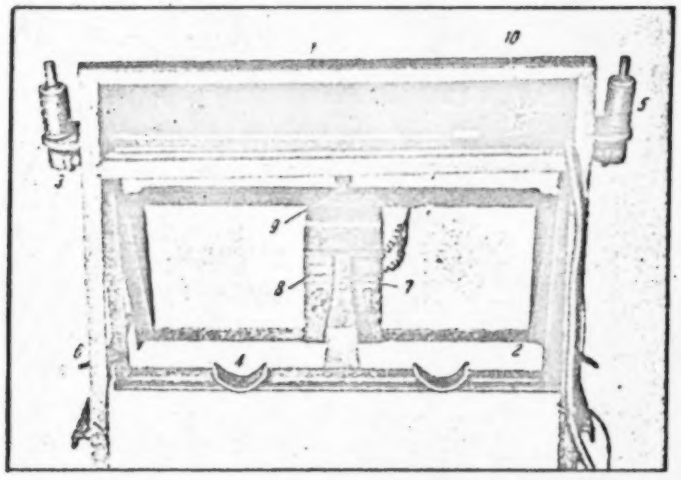

Fig. 1. General view of the ballistocardiogram recording setup. Description in text.

The indirect ballistocardiographs are easily calibrated accurately, which is undoubtedly one of their advantages. But they have also disadvantages, mainly their being cumbrous.

The direct ballistocardiograph was introduced to clinical practice later. Of the very numerous types, Smith's [3] direct ballistocardiograph transducer has been most used, this recording all three parameters simultaneously. This is achieved by integrating and differentiating the electromagnetic transducer output electronically, the transducer sensing the velocity.

A major disadvantage of the direct method is the difficulty or impossibility of calibration, as Kazmeier and Schild state. So in analyzing and evaluating the ballistocardiograms only the qualitative aspects are usually considered.

For recordings to be satisfactory the natural frequency of the platform or transducer must be higher than that of the body, if distortion due to resonance and beats is to be avoided. Starr's table has a satisfactory frequency characteristic but has a second degree of motional freedom, which may affect the curve shapes.

The direct transducers also have this second degree of freedom. Also the various transducers vary in weight and in envelope elasticity, which introduces additional factors into the determination of the frequency characteristics of the transducer plus patient system.

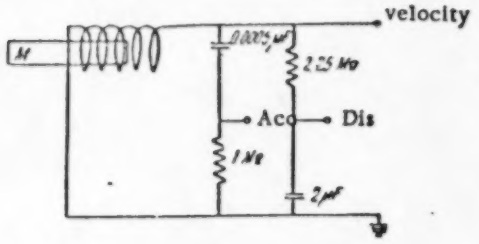

Fig. 2. Circuit of the integrating and differentiating device.
Description in text.

Our ballistocardiograph (Fig. 1) is in essence an attempt to combine the advantages of the indirect mechanical recording system with Smith's direct ballistocardiograph. The mechanical system is based on the free-bob pendulum principle, giving a high natural frequency and simultaneously minimizing the second degree of freedom. The pendulum system is convenient also in being easily calibrated.

The mechanical system is comprised of the following parts:

The angle-iron frame and stand (1) fixed on a solid couch. The bob frame (2) is also of angle-iron, the upper cross-bar being freely supported in ball-bearings fixed to the brackets (3) in the stand. The lower cross-bar carries two shaped rests for the lower parts of the patient's legs, (4). The stand carries two vertical adjustment screws (5) attached to the bob frame to adjust the frame relative to the couch (for leg thickness).

The sides of the stand carry catches (6) to retain the bob frame in the central position. These can also be used for calibration purposes (the catches have gaps within which the frame can be moved over a strictly defined range). The ballistocardiograms can then be calibrated by adjusting the sensitivity.

The electrical circuit comprises a magnet attached to the lower cross-bar in the bob-frame by the brass rod (7) and a coil (8) of 100,000 turns of copper wire 0.08 mm in diameter. The coil, being fixed to stand, can be adjusted vertically by the screw (9) to follow the bob frame, so the magnet moves freely within the coil; the capacitors and resistors (10) for differentiating and integrating the signal (Fig. 2) are mounted in the upper part of the stand.

This ballistocardiograph is simple to build, portable and cheap.

SUMMARY

A new model of ballistocardiograph was suggested, in which the advantage of the mechanical system of indirect recording is used in conjuction with the principle of direct ballistocardiography according to Smith's method. The mechanical system of this apparatus is designed by the principle of a pendulum with a free pendant. This provides high frequency of oscillations of the apparatus and simultaneously it causes the second degree of free movement to come down to a minimum. The system of pendulum is, likewise, convenient since it is easily calibrated.

The ballistocardiograph which is proposed is easily handled, portable and inexpensive.

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DETERMINATION OF SMALL AMOUNTS OF HISTAMINE IN BIOLOGICAL FLUIDS FROM ALLERGIC SKIN REACTIONS

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A prominent place is afforded to the elucidation of the role of histamine and of substances with a histamine-like action in the experimental and clinical study of the pathogenesis of allergic reactions. The authors of this paper have been required to determine very small amounts of such substances. The methods generally used do not, however, permit of the detection of such small amounts. Thus one of the most sensitive methods, depending on the reaction of isolated guinea pig intestine, permits of the detection of not less than 0.005 mg of histamine in a given solution (L. A. Oivin and V. I. Oivin). We have found that such doses of histamine do not cause any change in the blood pressure of cats.

Change in the rate of flow of blood through renal vessels of a dog after introduction of skin dialyzate from a nettle-rash patient.

In searching for a method enabling us to detect very small amounts of histamine, we directed our attention to the very high sensitivity to histamine of the renal blood vessels. These are dilated by histamine in dilutions of $1:10^{-6}$ to $1:10^{-7}$ which have no perceptible effect on systemic pressure. This leads to an increased rate of flow through the renal blood vessels. We measured the rate of flow by means of a Rein diathermic flow-meter. A special electrode is placed on an exposed, but not opened, artery, and the blood flowing through it is heated by means of a high frequency current. This gives rise to a temperature difference between the blood distal and proximal to the electrode. The faster the blood flow, the less time it spends in the high frequency field, and the smaller is the temperature difference. This temperature difference is picked up by a thermocouple mounted into the electrode, and the readings are recorded through a galvanometer on a moving photokymograph strip. Simultaneously with the measurement of blood flow, we registered blood pressure, under Hexenal anesthesia.

by means of an optical manometer. The electrode of the Rein flow-meter was inserted through an abdominal incision, and placed on the renal artery. The test solution was introduced into the iliac vein. The presence of histamine or of a histamine-like substance was indicated by increase in the rate of flow through the renal artery.

We tested skin dialyzates from normal controls (9 tests) and from patients suffering from nettle-rash, taken from unaffected parts of the skin (4 specimens) and from the urticarial wheals (10 specimens). The skin dialyzates were prepared in the following way. A glass cup, with a base diameter of 38 mm, covering a skin area of 10.33 cm^2, was fastened to the selected surface with an elastic tape. The cup was filled through an opening at its top with 6 ml physiological saline, which remained in contact with the skin for 30 minutes, after which it was pipetted out. The dialyzate thus obtained was taken for injection with no further treatment. The amount injected for test was 1-3 ml. Apart from this, we made a number of tests of edema fluid, aspirated from the wheals by means of a fine needle. For purposes of comparison, we determined the action of 1 ml of histamine solutions, in dilutions of 1:10,000 and 1:10,000,000. In all, we performed 28 tests.

Our tests showed that the dialyzates from freshly erupted urticarial wheals contain substances which increase the rate of flow of blood in the renal artery, without affecting the systemic blood pressure. This effect was absent from material from healthy controls and from the unaffected parts of the skin of nettle-rash patients. Tests conducted with standard solutions of histamine showed that an effect of the same order as that given by the urticarial dialyzates was given by dilutions of $1:10^{-7}$.

Substances exerting a histamine-like action on the artery were detected in the edema fluid aspirated from the wheals. This finding confirms the view that urticarial wheals are the result of an angioneurosis, due to the histamine-like action of the edema fluid.

SUMMARY

A method of determination of histaminic activity in biological fluids (corresponding to contents of histamine equal to $1:10^{-7}$) is described. This method consists of determination of the changes of velocity of the renal blood flow under the effect of histamine in dogs. The vascular reaction is registered by the change of blood flow velocity with the aid of Rein's flow-meter. The data obtained verify the presence of histaminic activity in the fluid derived from urticarial vesicles.

LITERATURE CITED

[1] I. A. Oivin and V. I. Oivin, Vestnik. Venerol. i Dermatol., No. 6, pp. 12-15 (1949).